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Paradoxical Effect of Hypercapnia on Toad Heart Muscle

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With the technical assistance of María A. Tau de Tau

SUMMARY **Experiments were performed on strips dissected from toad ventricles driven at a constant frequency of 12 beats/min. After equilibration, the P_{CO_2} of the medium was altered from 25 to 95 mm Hg or from 95 to 25 mm Hg. Developed tension (DT) and maximal rate of tension development (dT/dt_{max}) were recorded during a 30-minute period after the change in P_{CO_2}. In the first experimental series at 30°C, increasing P_{CO_2} resulted in a decrease in DT and dT/dt_{max}, followed by a recovery of contractility that reached levels higher than controls. In the second and third series, performed after the addition to the bath of practolol (1×10^{-6} M) or after reserpinization, high P_{CO_2} depressed contractility but the recovery did not surpass control values after 30 minutes of hypercapnia. In this series, when high P_{CO_2} was replaced by low P_{CO_2} there was an increase in DT and dT/dt_{max} followed by a decrease that reached control levels within a 30-minute period. In the fourth series, at 22°C the significant decreases in DT and dT/dt_{max} observed after increasing P_{CO_2} were followed by a recovery that surpassed control values. These results define the existence in toad cardiac muscle of a mechanism that tends to return contractility to control levels after a change in P_{CO_2}. Within 30 minutes this mechanism, present even after inhibition of catecholamine action, completely counteracts the primary negative inotropic effect of high P_{CO_2} and the positive inotropic action of hypocapnia.**

EXPERIMENTS on cat papillary muscles[1-3] describe a biphasic effect of hypercapnic acidosis on myocardial contractility. After the P_{CO_2} of the medium is increased, a decrease in contractility is followed by a spontaneous and partial recovery. This recovery takes place in spite of persistent and severe hypercapnia. Although a participation of catecholamines in the biphasic response to P_{CO_2} could not be ruled out, the fact that in these previous experiments[3] the biphasic phenomenon appeared to be calcium- and temperature-dependent indicates that other mechanisms are involved in the recovery process. For example, there can be an increased availability of calcium ions. This calcium, derived either from intracellular stores or extracellular fluid, would then be available to overcome a response to calcium depletion at the level of myofilaments, such as might be due to intracellular acidosis.[4, 5]

Although a negative inotropic effect of high P_{CO_2} on frog ventricle has been established,[6] the transient phenomena described for mammalian myocardium have not been studied in the amphibian heart. Several lines of evidence[7-12] indicate that excitation-contraction coupling is different in amphibian and mammalian heart tissue. In frog cardiac tissue the sparse and less organized sarcoplasmic reticulum (SR)[7] suggests a possible important role for mitochondria in calcium release and sequestration during the contraction-relaxation cycle,[8] and data provided by voltage clamp[9-11] and other studies[12] suggest that sources other than SR provide calcium to activate the contractile proteins. For these reasons, toad ventricular muscle was chosen for the present study to further elucidate the mech-

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anism responsible for the biphasic effect of P_{CO_2} described for mammalian heart.

Methods

Experiments were performed on strips dissected longitudinally from toad (*bufo arenarum* Hensel) ventricular wall. The length of the preparations ranged between 10 and 12 mm and their largest diameters between 1.5 and 2.0 mm.

The methods used for mounting, stimulation, and recording were essentially identical to those used previously.[3] Briefly, ventricular strips were mounted vertically in chambers containing Ringer's solution equilibrated with a gas mixture of 97% O_2 and 3% CO_2. The composition of the Ringer's solution (mM) was: NaCl, 114.98; KCl, 3.20; $NaHCO_3$, 20.59; KH_2PO_4, 0.30; $MgSO_4$, 1.20; $CaCl_2$, 1.50; glucose, 13.

Isometric mechanograms were recorded on a Sanborn model 7100 oscillographic recording system and the first derivative of developed tension (dT/dt) was obtained by a resistance-capacitance (RC) differentiator (R = 51 KΩ and C = 0.047 μF). Contraction frequency was kept constant at 12 beats/min, in all the experiments. Ringer pH and P_{CO_2} were measured by the appropriate electrodes thermoregulated at the temperature of the experiment. In some experiments the pH was measured and the P_{CO_2} calculated by the Henderson-Hasselbach equation.

Three series of experiments were performed at a constant temperature of 30°C and a fourth series at 22°C. One of the experimental series at 30°C was performed after the addition of a single dose of practolol (1.10^{-6} M) 1 hour before the experiment started. This dose blocked the mechanical response to a catecholamine dose of 3.10^{-8} M. One hour after practolol administration, a significant depression of developed force (56.4 ± 8.6% of control) and in maximal rate of tension development (dT/dt_{max}) (58.2 ± 11.7% of control) was observed. Another series was performed at 30°C with ventricular strips from toads which had received intraperitoneal reserpine (10 mg/animal) 24 hours prior to death. Catecholamine depletion was tested by increasing the intensity of the stimulus 2 to 4 times. No changes in developed tension or dT/dt_{max} were elicited by this maneuver. Table 1 shows values of resting and developed tension obtained after the stabilization period at a P_{CO_2} of about 25 mm Hg. In all cases the general experimental procedure was that previously described:[3] the P_{CO_2} of the medium was increased, after a stabilization period, to approximately 95 mm Hg, and developed tension and dT/dt were continuously recorded during a 30-minute period. The pH and P_{CO_2} were measured every 3 minutes. In the experiments performed with the β-blocking agent and on ventricular strips from reserpine-pretreated toads, the low P_{CO_2} gas mixture was reintroduced after the hypercapnic period. Developed tension and dT/dt_{max} were recorded during the following 30 minutes and values of pH and P_{CO_2} obtained every 3 minutes.

For statistical analysis the values of tension and dT/dt_{max}, expressed as percent of control, were obtained at the moment of maximum depression and 30 minutes after the P_{CO_2} change (during the hypercapnic period); or, conversely, at the moment of maximum enhancement of contractility and after 30 minutes of hypocapnia. In this way, the mean minimal percent value obtained during the exposure to high P_{CO_2} or the mean maximal percent values during the exposure to low P_{CO_2} were compared with each other and with the control. For the hypercapnic period, the "degree of depression" (Δd), of tension and dT/dt_{max} was defined as the mean difference between control values and values obtained at the point of maximal depression. The "degree of recovery" (Δr) was taken as the mean difference between the values reached after 30 minutes, and the minimal values of a given series. Mean rate of depression ($\overline{Rd}$) and mean rate of recovery ($\overline{Rr}$) are the mean of the data obtained by dividing Δd and Δr in each experiment by the time during which these changes occurred. Under hypocapnic conditions, the "degree of enhancement" (Δe) of tension and dT/dt_{max} was defined as the mean difference between control values and the values obtained at the point of maximal enhancement of contractility. The degree of subsequent decrease (Δsd) was the mean difference between values of maximal enhancement and values obtained at minute 30. Mean rate of enhancement ($\overline{Re}$) and mean rate of subsequent decrease ($\overline{Rsd}$) were obtained by dividing Δe and Δsd in each experiment by the time during which these changes occurred. The statistical analysis was carried out using independent samples and Student's test was used to evaluate differences. A P value less than 0.05 was considered significant.

Results

EFFECT OF HYPERCAPNIA ON THE ISOMETRIC MECHANOGRAM AT 30°C

Figure 1 shows the typical response of developed tension, dT/dt, and pH to changing the P_{CO_2} of the medium from 30 mm Hg to 99 mm Hg. An increase in the P_{CO_2} of the medium resulted in a decrease in tension and dT/dt_{max} followed by a spontaneous recovery that reached levels of contractility higher than the control.

In Figure 2A the overall results of this series are depicted. Values of mean tension and dT/dt_{max} at the point of maximum depression and after 30 minutes of hypercap-

TABLE 1 *Mean Values of Resting Tension, Developed Tension, and dT/dt_{max} Obtained at Low P_{CO_2}*

	n	RT(g)	DT(g)	dT/dt_{max}(g/sec)
30°C	6	0.34 ± 0.09	1.69 ± 0.20	8.00 ± 1.46
30°C, practolol	7	0.59 ± 0.07	0.83 ± 0.09	3.52 ± 0.32
30°C, reserpine	8	0.51 ± 0.08	1.10 ± 0.15	4.26 ± 0.64
22°C	7	0.59 ± 0.16	1.50 ± 0.20	4.29 ± 0.67

Values represent mean ± SE; n = number of experiments; RT = resting tension; DT = developed tension; and Dt/dt_{max} = maximal rate of tension development.

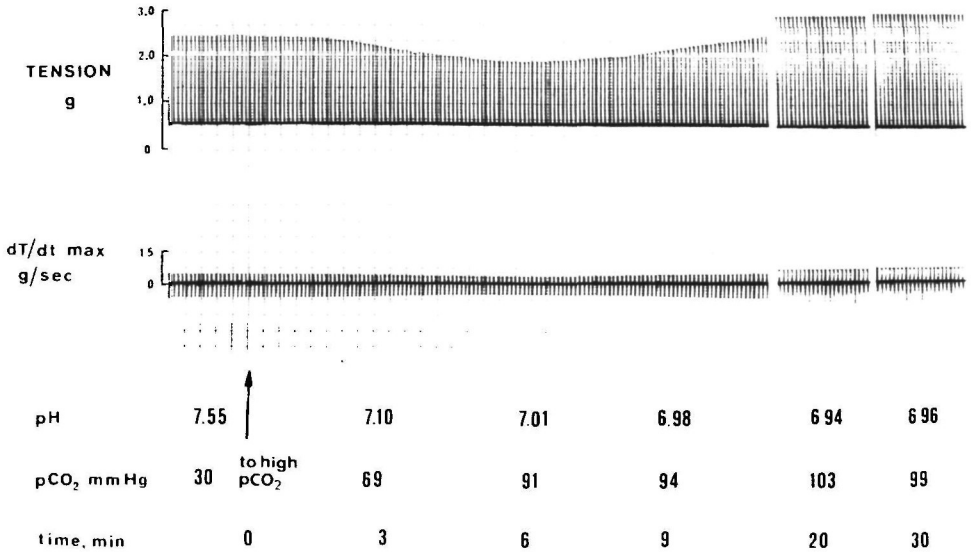

FIGURE 1 *Records of developed tension, dT/dt, pH, and P_{CO_2} values obtained at low P_{CO_2} (30 mm Hg) and during a 30-minute period of hypercapnia.*

nia are expressed as percent of control. Mean pH and P_{CO_2} values and mean time at which maximal depression was reached are also presented in this figure. A slight but significant decrease in contractility occurred after the P_{CO_2} change. This was followed by a recovery which surpassed control values. Table 2 summarizes the results of this and the following series.

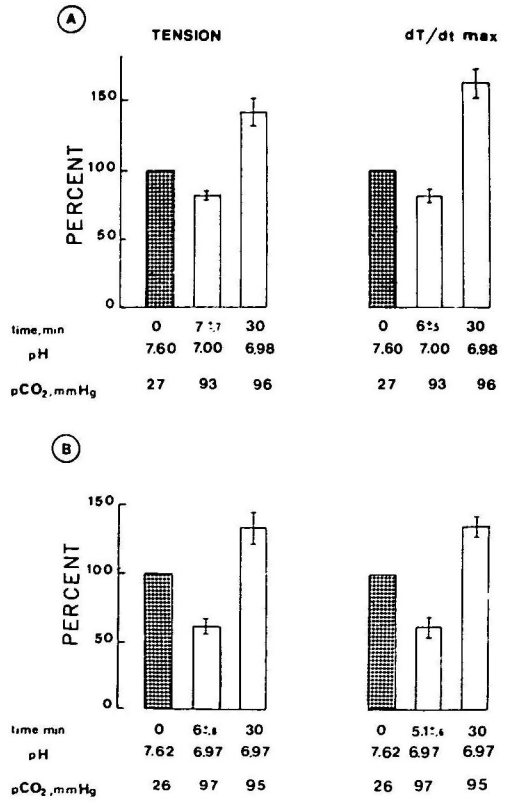

FIGURE 2 *Effect of a 30-minute period of hypercapnia on tension, dT/dt_{max}, pH, and P_{CO_2} values obtained at 30°C (A) and 22°C (B). In these figures and in Figures 3A and 4A we have depicted mean tension and dT/dt_{max} values only at the point of maximal depression, and after 30 minutes of hypercapnia.*

INFLUENCE OF CATECHOLAMINES

Addition of Practolol

Similar experiments were performed after the addition of practolol (1×10^{-6} M) to the bath. As shown in Figure 3A and Table 2 the biphasic effect of P_{CO_2} occurred in the presence of this β-blocking agent. However, the final levels of contractility obtained after the 30-minute period of hypercapnia were not significantly different from their own controls. In addition, the degree of depression of contractility was significantly higher, and the mean final points lower, than without practolol. (The difference in the final points was not significant for developed force.) Time to attain maximal depression was lower (statistically significant for developed tension) than in the experiments performed without β-blockade. The same was true for the degree and mean rate of recovery (significantly different for dT/dt_{max}). Therefore, the primary negative inotropic effect of high P_{CO_2} was more evident and the subsequent recovery of contractility was partially abolished by practolol.

Figure 3B shows the results of this series after the change from hypercapnia to hypocapnia. The "reverse" recovery can be seen under these conditions, i.e. a significant increase in contractility occurred with its maximal values after exposure for about 13 minutes to low P_{CO_2}, followed by a decrease in contractility that returned to control levels after 30 minutes of hypocapnia.

Pretreatment with Reserpine

As shown in Figure 4A and B and Table 2, results very similar to those obtained with practolol were found in this series: the pretreatment with reserpine augmented the maximal decrease in contractility produced by hypercapnia. Furthermore, the recovery was partially abolished.

When the muscles were returned to low P_{CO_2} the "reverse" recovery that had been observed with practolol was enhanced under this experimental situation: after having attained maximal levels, developed tension and dT/dt_{max}

TABLE 2 *Changes in Toad Cardiac Contractility during a 30-Minute Period of Hypercapnia*

	Tension				dT/dt_{max}			
	30°C	Practolol, 30°C	Reserpine, 30°C	22°C	30°C	Practolol, 30°C	Reserpine, 30°C	22°C
Min. Val. (% of control)	82.6 ±2.9*	66.4 ±4.2*	63.4 ±4.0*	61.9 ±4.9*	83.6 ±4.1*	65.3 ±2.1*	58.1 ±2.8*	60.4 ±7.5*
Δd (% of control)	17.4 ±2.9†	33.6 ±4.2†	37.0 ±4.0†	38.1 ±4.9†	16.4 ±4.1†	34.7 ±2.1†	41.9 ±2.8†	39.6 ±7.5†
Time (min)	7.0 ±0.7	4.0 ±0.8	6.0 ±1.3	6.0 ±0.8	6.1 ±0.5	4.0 ±0.3	6.0 ±1.6	5.1 ±0.8
$\overline{Rd}$ (% of control/min)	2.48 ±0.49	8.71 ±1.36	8.27 ±2.07	7.29 ±1.49	3.20 ±1.08	6.67 ±1.60	7.09 ±1.21	8.38 ±2.11
FP (% of control)	145.4 ±11.9*‡	117.7 ±18.8‡	121.3 ±14.9‡	136.1 ±11.2*‡	168.5 10.9*‡	107.8 ±13.2‡	124.4 ±10.9‡	137.8 ±5.6*‡
Δr (% of control)	62.5 ±13.2†	49.9 ±17.3†	59.6 ±17.6†	74.1 ±12.4†	84.7 ±8.3†	45.5 ±13.6†	66.4 ±12.6†	77.4 ±10.4†
$\overline{Rr}$ (% of control/min)	2.70 ±0.58	1.89 ±0.50	2.30 ±0.72	3.01 ±0.47	3.32 ±0.37	1.72 ±0.51	2.80 ±0.54	3.04 ±.39

Values represent mean ± SE expressed as percent of control.
Min. Val. = minimum values; Δd = degree of depression; t = time to attain maximal depression value; $\overline{Rd}$ and $\overline{Rr}$ = mean rates of depression and recovery; FP = final point attained after a 30-minute period of hypercapnia; Δr = degree of recovery; Δt = time in which the degree of recovery takes place.
* Significantly different from the control.
† Significantly different from zero.
‡ Significantly different from the maximal depression.

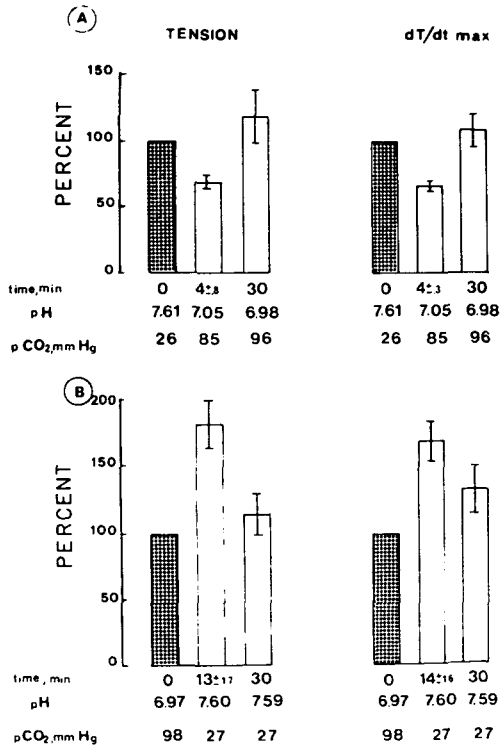

FIGURE 3 *A: effect of 30 minutes of hypercapnia on tension, dT/dt_{max}, pH, and P_{CO_2} values; overall results of the experiments performed after proctolol administration of 1.10^{-6}M. B: effect of 30 minutes of hypocapnia on tension, dT/dt_{max}, pH, and P_{CO_2} values; overall results of the experiments performed after the addition of practolol, 1.10^{-6}M. In this figure and Figure 4B, we have shown mean tension and dT/dt_{max} values (± standard errors) only at the point of maximal enhancement of contractility and after 30 minutes of hypocapnia. Note that in this series, as in the one shown in Figure 4B, control values represent values obtained after a 30-minute period of profound hypercapnia.*

returned to values significantly lower than the control ones after 30 minutes of hypocapnia.

EFFECT OF TEMPERATURE

Figure 2B and Table 2 depict the result of this series. As shown, there was a significant depression of contractility

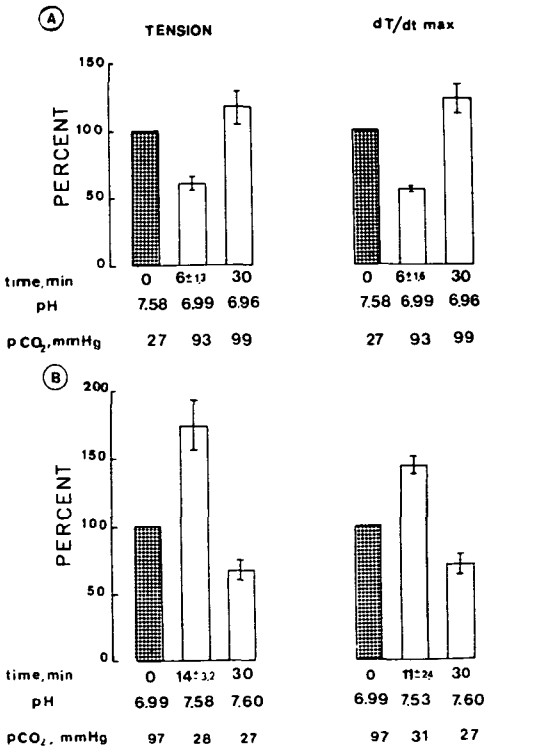

FIGURE 4 *Effect of 30 minutes of hypercapnia (A) and of 30 minutes of hypocapnia (B) on tension, dT/dt_{max}, pH, and P_{CO_2}; average values of all the experiments performed in muscles from reserpinized toads.*

followed by a recovery that, at 30 minutes, significantly exceeded control values. The degree and mean rate of depression at 20°C were significantly higher for developed tension and dT/dt_{max} than at 30°C. Mean final point at 30 minutes was significantly lower for dT/dt_{max} than at the higher temperature. The degree of recovery and mean rate of recovery were similar at both temperatures.

Discussion

The present experiments have shown that increasing the P_{CO_2} of the medium resulted in a slight decrease in contractility in toad cardiac muscle that is followed by a recovery that significantly surpasses control levels after 30 minutes of hypercapnia. When the action of catecholamines was prevented by β-blockade or depletion of catecholamine stores by prior reserpinization, the initial depression of contractility increased significantly and the subsequent recovery was partially but not totally abolished. A similar augmentation of the initial decrease in contractility and depression of the recovery phase was observed at 22°C. However, under these conditions, developed force and dT/dt_{max} surpassed control levels at the end of the hypercapnic period. A "reverse" recovery could be produced by changing from high to low P_{CO_2} in the experiments with practolol and in reserpinization i.e., after a 30-minute period of severe hypercapnia the introduction of low P_{CO_2} results in an increase in developed tension and dT/dt_{max}, followed by a decrease in both parameters that at the end of the hypocapnic period equaled the control values. The well known deleterious effect of high P_{CO_2} as well as the enhancement of contractility during hypocapnia[6, 13–18] were seen in our experiments in toad heart muscle. However, within the 30 minutes that follow the P_{CO_2} change, the ensuing recovery and "reverse" recovery of contractility were so great in this tissue that they resulted in an increased contractility of the hypercapnic heart and, conversely, a decreased DT and dT/dt_{max} in the hypocapnic cardiac tissue.

Following a similar line of thought for the mammalian heart[3] the biphasic effect of P_{CO_2} could be conceived and analyzed as the mechanical resultant at every moment of two different processes: a primary P_{CO_2} effect and a triggered secondary mechanism tending to return contractility to control values. A greater decrease in contractility with a depressed recovery phase was seen when catecholamine action was prevented, indicating that the recovery mechanism was somewhat depressed. However, the fact that the transient recovery also occurred after catecholamine depletion or β-blockade strongly suggests that other mechanisms are involved in the recovery process. This conclusion is also supported by the biphasic effect of hypocapnia described in the experiments performed with catecholamine-depleted tissue and with practolol.

The fact that the recovery and the "reverse" recovery mechanisms were so important in toad heart muscle as to abolish completely both the primary deleterious effect of high P_{CO_2} and the positive inotropic effect of hypocapnia, even when catecholamine action was prevented, might be interpreted as an enhancement of the trigger mechanism that in mammalian heart tended to return contractility toward control values.[3]

One possible clue to the nature of this trigger mechanism was given by the experiments performed at 22°C. These results suggest that an active process, which is depressed at the lower temperature, may also play a role in the recovery and reverse recovery phase of this biphasic phenomenon. Previous experiments on cat papillary muscles[3] showing the dependence of the biphasic response to P_{CO_2} on external calcium concentration led to the tentative hypothesis that there is an increased availability of calcium ions in response to a calcium depletion at the level of myofilaments such as might be due to intracellular acidosis.[4, 5] Since it has been shown[4] that the affinity of SR for calcium increases in an acidotic medium this calcium might more probably derive from extracellular fluid or from intracellular stores other than SR. Evidence showing that amphibian cardiac tissue possesses a sparse SR,[7] that its contraction is greatly dependent on extracellular calcium,[9–12] and that mitochondrial calcium sequestration and release is of importance in the contraction-relaxation cycle[8, 19] suggest that the present results would be in keeping with this hypothesis.

In attempting to explain the underlying mechanisms for the recovery phenomenon other factors, in addition to those mentioned above, should be considered. For instance, knowledge of the buffer capacity of toad ventricular muscle is required. In any case, some recovery mechanism must exist in toad cardiac muscle that is able to return contractility to a higher than control level after it reaches a nadir in the presence of sustained hypercapnia.

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Rate-Dependent Changes in Extracellular Potassium in the Rabbit Atrium

DIANA L. KUNZE

SUMMARY **We measured levels of potassium ion in the extracellular space of isolated superfused rabbit atria continuously with double-barreled microelectrodes of which one barrel was a K^+ liquid ion-exchanger microelectrode and the other a potential-sensing micropipette. Increases in heart rate resulted in transient increases in extracellular potassium ($[K^+]_0$). When the quiescent atrium was stimulated the maximal increase was 0.4 mM at rates of 60/min, 0.7 mM at 90/min, 0.9 mM at 120/min, 1.3 mM at 200/min, and 1.8 mM at 300/min. The increase was not sustained during continued stimulation but declined toward prestimulation levels. When the stimulus was terminated the extracellular potassium activity decreased below bathing solution values by 0.2 mM after 60/min, 0.5 mM after 90/min, 0.7 mM after 120/min, 0.9 mM after 200/min, and 1.0 mM after 300/min and subsequently returned to a value equal to that of the bathing solution. The magnitude of the decline in extracellular potassium activity during prolonged stimulation was markedly decreased when the bathing solution contained either zero potassium, ouabain, LiCl, or a decreased P_{O_2} such that an elevation in $[K^+]_0$ persisted during stimulation. Moreover, the reduction in $[K^+]_0$ that followed the cessation of stimulation also was inhibited. These results support a role of the Na-K pump in maintaining extracellular potassium activity during changes in cardiac rate.**

AS CARDIAC rate is increased there is a transient loss of potassium from isolated preparations of cardiac tissue,[1, 2] from whole heart preparations in vitro,[3-5] and from the heart in vivo.[6] Although there is a difference of opinion among various investigators about the magnitude of unidirectional fluxes during rate changes, there is general agreement that a net efflux of potassium occurs over the first few minutes after a rate increase.[7] This loss probably reflects the outflow of potassium in excess of inflow which contributes to the repolarization phase of the cardiac action potential. If there is a restriction to diffusion away from the cell membrane, a net potassium efflux would be expected to increase extracellular levels of potassium and establish a gradient between the extracellular space and the perfusion fluid. Recently Kline and Morad[8] used potassium-sensitive electrodes to study frog ventricular tissue and showed that potassium activity increased by as much as 1 mM in the extracellular space of frog ventricular muscle in response to a single action potential. Potassium accumulated with successive action potentials, and $[K^+]_0$ subsequently decreased after the period of stimulation. The magnitude of accumulation and the time course of decay following stimulation were dependent on the diameter of the strip and the depth of penetration of the electrode as would be expected in a superfused preparation with varying diffusion distances to the bathing solution (i.e., with varying thickness of unstirred layers).

Because the resting potential of the cardiac cell is influenced by the extracellular potassium activity,[9] any changes in potassium distribution must be considered in evaluating the normal pattern of electrical activation and inactivation. This study was undertaken to examine the effects of prolonged stimulation on the extracellular potassium activity. The present report deals with the effect of changes in cardiac rate on extracellular potassium activities in isolated superfused rabbit tissue studied with a double-barreled microelectrode, one barrel of which was a K^+ liquid ion-exchanger microelectrode and the other a potential-sensing micropipette. This electrode configuration is essential for these studies because the potential-sensing pipette indicates whether the electrode tip is in the extracellular space or in the intracellular fluid. Also, any extracellular voltage changes occurring during the cardiac action potential must be subtracted from the potassium electrode reading.

Methods

POTASSIUM-SENSITIVE ELECTRODES

The potassium electrode used in these experiments is a glass micropipette (one side of a double-barreled pipette)

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