Spontaneous Activity in Isolated Rabbit Atria Below 5°C. (32582)

E. T. ANGELAKOS* AND J. T. MAHER (Introduced by M. Landowne)
U. S. Army Research Institute of Environmental Medicine, Natick, Massachusetts and Boston University School of Medicine, Boston, Massachusetts

It is generally accepted that in nonhibernating species, the spontaneous activity of the heart ceases when the tissue temperature is lowered to 19°-15°C(1,2). By contrast, the cardiac pacemaker of hibernators continues to discharge at temperatures as low as 0°C. The latter can be shown in isolated heart preparations even when obtained from animals which were not in hibernation(3,4). Thus, a fundamental difference appears to exist in the cardiac pacemaker of hibernating and nonhibernating species in regard to its sensitivity to low temperatures.

Over the past two years, studies have been made at this institute with isolated right atria of the rabbit in attempts to discover conditions under which spontaneous activity of this preparation from a nonhibernator could be maintained at low bath temperatures. Recently we were successful in maintaining such preparations with spontaneous activity at temperatures below 5°C under certain conditions(5). Since, to our knowledge, this is the first time that this has been accomplished in a nonhibernator, a preliminary report of the key findings which may be of general interest seemed warranted.

Methods. The preparation used has been described in detail elsewhere(6). Briefly, isolated right atria of the rabbit were suspended in a chamber continuously perfused at a rate of 4 ml/min with a modified Locke's solution with the following concentrations (in mM/l): Na, 160; K, 5.6; Ca, 1.8; Cl, 164; HCO_3, 6; glucose 11. After equilibration at 30°C, the temperature was lowered at the rate of 1°C per 3 to 5 minutes by controlled cooling of the chamber jacket, while the temperature of the perfusate in the 20 ml chamber was monitored continuously with a thermistor probe (YSI series 400). Both the mechanical and electrical activities of the preparation were recorded with a Brush Mark 280 recorder. The former was measured as the isometric tension with a Grass FT03 force transducer. Electrical responses were recorded from the atrial surface with bipolar electrodes leading through a Tektronix 122 preamplifier. The mechanical response was used as the criterion for activity in these studies. The electrical potential was monitored solely to confirm the criterion of cessation of activity since electrical potentials may be present in the absence of measurable contractions. In each experiment, reproducible observations were made in no less than 5 and up to 20 preparations.

Each preparation was tested only once. It was cooled and the temperature of arrest was first noted while the preparation was perfused with a solution of a given K concentration. Arrest of spontaneous activity was established by observations of no less than 2 minutes. At this point, the neurohumors were added in the perfusion fluid and once spontaneous activity was resumed, the same preparation was cooled further until a new arrest temperature was reached. This permitted comparison of arrest temperatures before and after addition of the neurohumor in a given K concentration in the same preparation.

Results. Under control conditions, spontaneous activity of the atria ceased when the bath temperature was lowered in the range of 20-17°C. At this point, addition of acetylcholine (Ach) (10^{-6} g/ml) to the perfusion fluid resulted in resumption of spontaneous propagated activity within 0.5 to 3 minutes. These observations are in general agreement with previous reports(7,8,9). In the presence of Ach, the preparations continued to beat as the bath temperature was lowered further. But again, spontaneous activity ceased at temperatures in the range of 15.5 to 12.0°C.

The effects of K ion concentration and of catecholamines (norepinephrine and epinephrine) were investigated and the results are summarized in Table I. In preparations

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TABLE I. Temperature of Arrest of Spontaneous Propagated Pacemaker Activity (Mean ± S.E. in °C).

	N	Control	After Ach	Difference	After CA	Difference
		Experiments in 5.6 mM/l, K^+				
	7	18.9 ± .5				
	8	17.6 ± .9	13.6 ± .4	4.0 ± .7*		
	9	19.3 ± .6			10.2 ± .9	9.1 ± .6*
Total	(24)	18.6 ± .4				
		Experiments in 2.8 mM/l, K^+				
	6	14.5 ± .4				
	5	17.1 ± .8	13.2 ± .3	3.9 ± .5**		
	9	16.1 ± .9			4.2 ± .4	11.8 ± 1.1*
Total	(20)	15.9 ± .5				
		Differences, effect of K^+ concentration				
		2.8 ± .7†	0.4 ± .5	0.1 ± 1.0	6.0 ± .9†	2.7 ± 1.2††
		Differences, comparison of neurohumoral effects				
	Ach effect			4.0 ± .5		
	CA effect					10.5 ± .7
	Difference				6.5 ± .9†	

By paired data t test, * $p < .001$ ** $p < .005$
By non-paired data t test, † $p < .001$ †† $p < .025$

where the K concentration of the perfusion fluid used was 2.8 mM/l (reduced by one-half and replaced with an equimolar amount of Na), the temperature at which spontaneous activity ceased was lower by roughly 3°C as compared to the controls (Table I). Addition of Ach at this point, re-established activity, but again the preparations ceased to beat at temperatures above 12°C. However, when spontaneous activity had ceased in atria perfused with low K solutions, addition of catecholamine (CA) (10^{-6} g/ml) again resulted in re-establishment of spontaneous activity, but in this case the preparations continued to beat at temperatures as low as 2 to 5°C (Table I and Fig. 1). Spontaneous activity was maintained below 8°C for more than 2 hours in a few preparations where this was tested. Both epinephrine and norepinephrine were used in separate experiments, but the results were similar and were therefore combined in the Table. Similarly, a decrease in the temperature at which spontaneous activity was maintained was observed in atria perfused with the normal concentration of K when catecholamines were added following initial cessation of activity. However, in this case, the final temperatures reached were much higher (Table I).

Discussion. The significance of the above observations cannot be evaluated fully at the present. The temperatures at which spontaneous activity ceased in atria perfused with catecholamines and low K were the same as those observed in hearts of several hibernating species, *e.g.*, woodchucks, chipmunks(3). It has been suggested that in preparations from nonhibernators, arrest of spontaneous cardiac activity at low temperatures may be due to a decrease in membrane action potential producing initially a lack of propagated excitation and eventually inexcitability(4,9,10). In such a case, both Ach (with its well-known action to increase membrane permeability to K), and low external K ion concentration, would tend to increase membrane potential and hence maintain propagated excitation. However, the ionic basis of catecholamine effects on the pacemaker potential is not established. The present observations indicate that the effects of catecholamines and low K are additive in extending pacemaker activity at low temperatures. By contrast, the effects of Ach and low K are not additive. This suggests that the action of Ach is probably through a mechanism involving K, whereas the effect of catecholamines is dependent upon some other mechanism. In any event, the

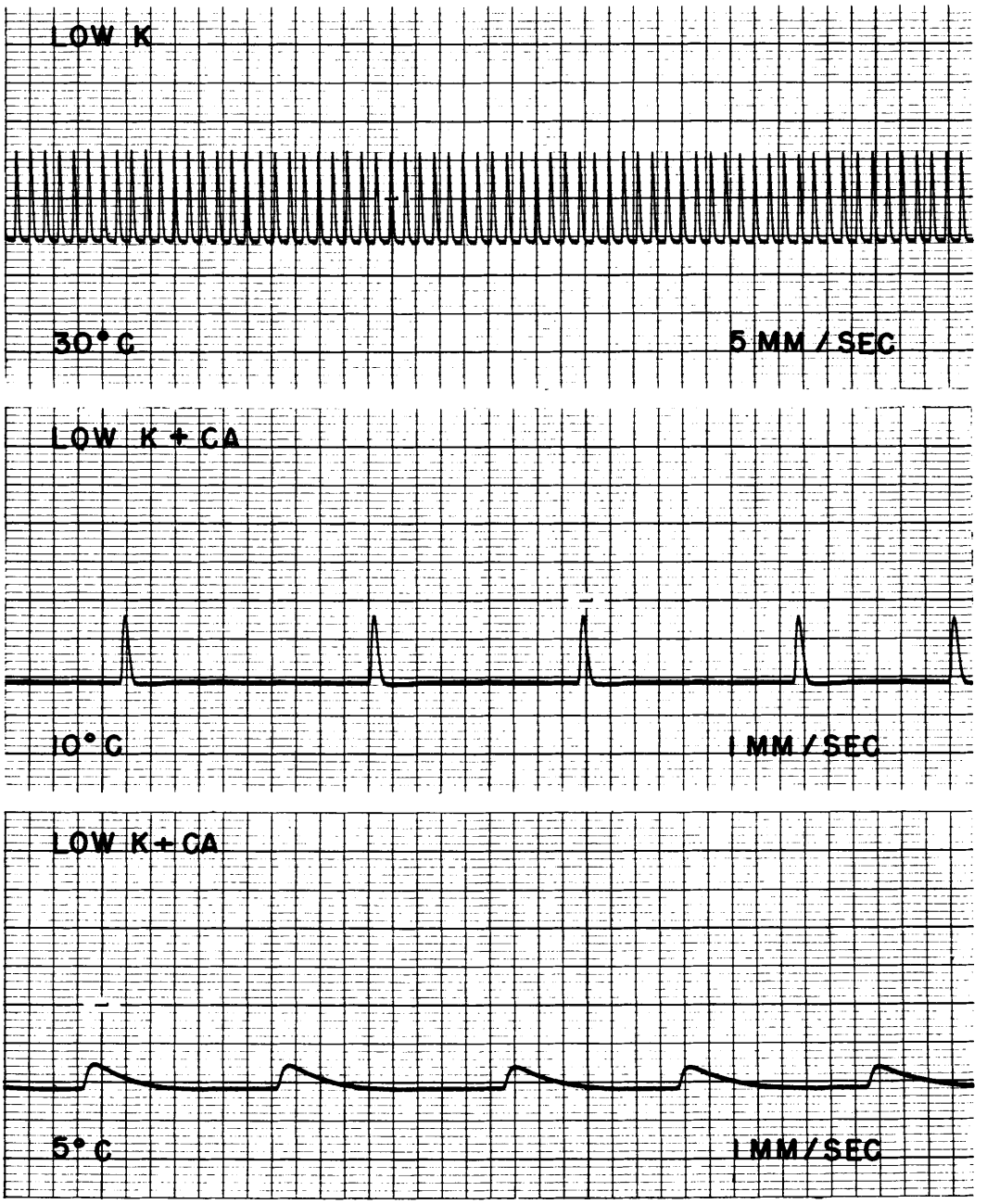

FIG. 1. Isometric contraction records of isolated rabbit atrium perfused with low potassium (K) (2.8 mM/l) and catecholamines (CA) 10^{-6} gm/ml). (Tension calibration: 0.1 g/division at 30°C and 0.25 g/division at 10° and 5°C).

present results in a nonhibernator indicate a need for studies to explore the possible role of adrenergic mechanisms and local cardiac catecholamine stores in the adaptation of the pacemaker of hibernating species to low temperatures.

Summary. Spontaneous propagated activity was maintained at low temperatures in rab-

bit atria with a combination of catecholamines and low potassium in the perfusion fluid. Under these conditions, atrial activity was present at temperatures below 5°C. Heretofore, spontaneous cardiac activity at such temperatures has been observed only in hibernating species.

1. Lyman, C. P., Chatfield, P. O., in The Physiology of Induced Hypothermia, Nat. Acad. Sci. Publ., 1956, v451, 80.

2. Hegnauer, A. H., Ann. N. Y. Acad. Sci., 1959, v80, 315.

3. Lyman, C. P., Blinks, D. C., J. Cell. Comp. Physiol., 1959, v54, 53.

4. Marshall, J. M., Willis, J. S., J. Physiol. (London), 1962, v164, 64.

5. Angelakos, E. T., Maher, J. T., (Abstract) The Physiologist, 1966, v9, 130.

6. Torchiana, M. L., Angelakos, E. T., Arch. Int. Physiol. Biochim., 1963, v71, 155.

7. Marshall, J. M., Vaughan Williams, E. M., J. Physiol. (London), 1956, v131, 186.

8. Johnson, E. A., Robertson, P. A., Brit. J. Pharmacol., 1957, v13, 304.

9. Torres, J. C., Angelakos, E. T., Am. J. Physiol., 1964, v207, 199.

10. Marshall, J. M., Circulation Res., 1957, v5, 664.

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Absorption Through Unstimulated and Secreting Canine Oxyntic Glandular Mucosa.* (32583)

HORACE W. DAVENPORT, WARREN S. REHM, AND BERGEIN F. OVERHOLT

Department of Physiology, The University of Michigan, Ann Arbor, Michigan and Department of Physiology and Biophysics, University of Alabama, Birmingham, Alabama

A substantial body of literature discusses rates of diffusion of substances across the oxyntic glandular mucosa. [Work through 1964 is summarized in(1); a more recent important paper is(2).] A major difficulty encountered when one tries to interpret data on diffusion through the gastric mucosa is that the area across which diffusion occurs is unknown. Do ions and compounds diffusing from lumen to blood cross only the surface epithelial cells, or, passing down the tubules, do they diffuse across the oxyntic and chief cells as well? Since the surface area of the glandular cells of the tubules (excluding the canaliculi) is 13 times the macroscopic area of the surface of the stomach(3), one's conclusion about the permeability characteristics of the membranes forming the barrier to diffusion are grossly different if one considers substances to diffuse only across the surface epithelial cells or to cross both the surface cells and those lining the tubules.

We have attempted to solve this problem by measuring rates of absorption of three compounds (ethanol, thiopental and salicylic acid) whose absorption is accomplished entirely by passive diffusion. These compounds are uncharged in the acid solution we used, and the electrical gradient across the mucosa has no influence on their absorption. We have measured their absorption by the unstimulated, non-secreting, and by the maximally stimulated, secreting mucosa.

The dimensions of the dog's oxyntic glandular mucosa have been measured by Canosa and Rehm(3), and Rehm, Schlesinger and Dennis(4) have calculated the rate of diffusion of a solute from the lumen into the pits and tubules. C_o is the concentration of solute in an essentially infinite reservoir of fluid on the surface of the mucosa, and C is the concentration of solute at any time and at any distance from the surface when the solute diffuses into an infinitely long column of solvent of uniform cross-section. If the velocity of flow of fluid in the column is zero, the ratio C/C_o for a solute (HCl) having a diffusion coefficient of 2.7×10^{-5} cm^2 sec^{-1} is 0.4 at a depth of 1.5 mm from the surface at the end of 600 sec [Fig. 4A, reference(4)]. The diffusion coefficient of ethanol is about 1×10^{-5} cm^2 sec^{-1}, and that

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