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Adrenaline: Mechanism of Action on the Pacemaker Potential in Cardiac Purkinje Fibers

Abstract. *The pacemaker potential in Purkinje fibers is generated by a slow fall in potassium current which allows the inward background currents to depolarize the membrane. Adrenaline shifts the relation between activation of the potassium current and membrane potential in a depolarizing direction. Consequently, during the pacemaker potential, the potassium current falls more rapidly to lower values and the inward currents then depolarize the membrane more quickly. The shift in the potassium activation curve produced by adrenaline is large compared to that produced by calcium ions. The molecular action of adrenaline may involve either a large change in the surface charge of the membrane or a change in the dependence of the potassium permeability on the local electric field.*

Several attempts have been made to determine how adrenaline accelerates the cardiac pacemaker, but most results have been negative. Adrenaline does not greatly influence the resting conductance or the sodium conductance involved in generating the action potential (*1*, *2*). However, the voltage-clamp technique has revealed the changes in time- and voltage-dependent current which underlie the pacemaker potential in Purkinje fibers (*3*–*5*). We have studied the action of adrenaline on these current changes.

The major time-dependent change in the pacemaker potential is a decrease in potassium current following repolarization at the end of the action potential (*3*–*6*). This fall in potassium current allows the inward background currents to depolarize the membrane to the threshold for initiating the action potential. Quantitative analysis of this potassium current (called i_{K_2} to distinguish it from i_{K_1}, which does not appear to be time-dependent) has shown that it is determined by a permeability variable, s, which is similar to the Hodgkin-Huxley permeability variables (*5*). Thus

$$i_{K_2} = s \cdot \overline{i_{K_2}} \qquad (1)$$

where $\overline{i_{K_2}}$ is the current when $s = 1$, and s obeys first-order kinetics:

$$ds/dt = \alpha_s(1 - s) - \beta_s s \qquad (2)$$

where α_s and β_s are functions of membrane potential (E) which have been determined empirically (*5*).

Adrenaline accelerates the pacemaker depolarization of spontaneously beating Purkinje fibers (*1*, *2*, *7*). To determine the mechanism of this action, we have studied the behavior of s during superfusion of a sheep Purkinje fiber with a Tyrode solution containing 5×10^{-7} g of adrenaline per milliliter (Fig. 1). The membrane potential was voltage-clamped at -80 mv, and the current changes following step depolarizations and hyperpolarizations were recorded. The current peak soon after return to -80 mv gives a measure of the degree of activation, s, since $\overline{i_{K_2}}$ is constant when E is constant. Equation 1 then becomes

$$i_{K_2} \propto s \qquad (3)$$

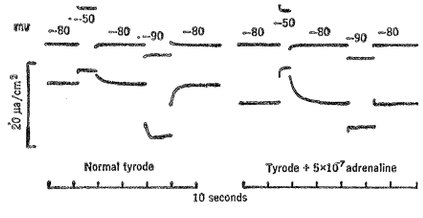

Fig. 1. Effect of adrenaline on voltage-clamp currents in sheep Purkinje fiber. (Left) Normal Tyrode solution. (Right) Tyrode solution containing 5×10^{-7} g of adrenaline per milliliter. Each record shows current (bottom) in response to 30 mv depolarization and 10 mv hyperpolarization from a holding potential of -80 mv. Note that in adrenaline solution no current is deactivated by hyperpolarization and that steady-state current at -80 mv is more negative than in absence of adrenaline. This change in steady-state current is attributable to shift in s_∞ (E) relation (see Fig. 2). Results shown in Fig. 2 were obtained by measuring current immediately following return to -80 mv after long-lasting depolarizations and hyperpolarizations.

Figure 2 shows the variation in the steady-state value of s (that is, s_∞) with membrane potential measured in terms of i_{K_2} at -80 mv. The major effect of adrenaline is to shift the curve in the depolarizing direction by about 30 mv. The total amplitude of the curve is also reduced, but it is not yet certain whether any significance can be attached to this effect. Pronethalol (10^{-6} g/ml) restores the curve to its original position. An intermediate concentration of pronethalol (5×10^{-7} g/ml) shifted the curve by about 15 mv.

Figure 3 shows measurements of the rate of change of potassium current as a function of the membrane potential, measured in terms of the reciprocal of the time constant of current change,

$$\tau_s^{-1} = \alpha_s + \beta_s \qquad (4)$$

The symbols are the same as in Fig. 2. Adrenaline also shifts this curve in the depolarizing direction, and at -90 mv, which corresponds to the beginning of the pacemaker potential, the change in current is much faster when adrenaline is present than when it is absent or when pronethalol is applied.

Thus, adrenaline has the same effect on the properties of i_{K_2} as a hyperpolarization. A simple way in which it might do this is to alter the local electric field controlling the s kinetics, perhaps by adding its own positive charge to the surface of the membrane. However, adrenaline might also act in some more indirect manner, for example, by chemically altering the state of the membrane so that the energy levels of the s reaction are changed. At present, we cannot decide between these and various other possibilities. It is worth noting, however, that the effect of adrenaline in shifting the relation between s_∞ and membrane potential is much greater

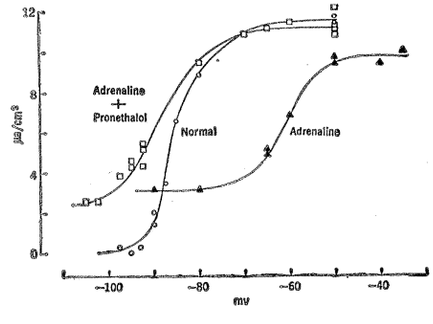

Fig. 2. Relations between steady-state degree of activation (s_∞) of slow K current and membrane potential, measured in terms of current immediately following return to -80 mv (see Fig. 1); ○, normal Tyrode; ▲, adrenaline (5×10^{-7} g/ml); and □, pronethalol (10^{-6} g/ml).

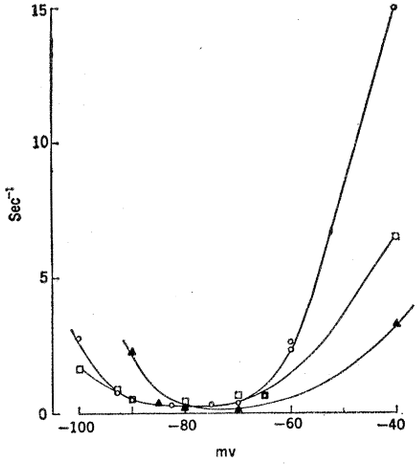

Fig. 3. Voltage dependence of rate of change of slow potassium current measured in terms of reciprocal of time constant (τ_s^{-1}) of current change. Symbols are the same as in Fig. 2.

than that of increasing the calcium ion concentration. Thus, increasing the external calcium concentration from 1.8 m*M* to 7.2 m*M* produces a shift of only 8 mv.

The physiological importance of these results lies in the fact that they account for the action of adrenaline in accelerating the pacemaker depolarization. At the beginning of the pacemaker potential the potassium current will decline more quickly, since the rate of change of i_{K_2} is faster in the presence of adrenaline (Fig. 3). Moreover, since the steady-state relation between *s* and membrane potential is shifted in the depolarizing direction (Fig. 2), i_{K_2} will fall toward a smaller value and so accelerate the depolarization throughout the pacemaker potential. Computer calculations have been done which show that these effects are quantitatively adequate to account for the chronotropic action of adrenaline.

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12 July 1968

Ethanol Oxidation by Hepatic Microsomes: Adaptive Increase after Ethanol Feeding

Abstract. *Hepatic microsomes contain an ethanol-oxidizing system distinct from alcohol dehydrogenase. In vitro, it has characteristics comparable to those of microsomal drug-detoxifying enzymes and, in vivo, it is capable of adaptation to the administration of ethanol. The existence of this microsomal ethanol-oxidizing system may explain ultrastructural, pharmacological, and biochemical effects of ethanol.*

Ethanol administration, in man given a variety of diets (*1*) and in rats fed either an adequate (*2*) or a deficient diet (*3*), produced proliferation of the smooth endoplasmic reticulum (SER) of the liver. This proliferation was accompanied by an increase in the activity of various hepatic microsomal drug-detoxifying enzymes (*3*). These structural and functional changes are very similar to those produced by a variety of pharmacologic agents (*4*). The concept that ethanol affects the SER by acting as other drugs do produced the following difficulty. Most substances that induce microsomal drug-detoxifying enzymes are metabolized, at least in part, in the microsomes (which comprise the SER) (*4*). The hepatic enzyme responsible for ethanol oxidation is thought to be alcohol dehydrogenase (ADH) (*5*), which is located in another cell compartment, the cell sap or cytosol (*6*). This problem prompted our study of ethanol-oxidizing activity in various subcellular fractions of the rat liver.

Ten male Sprague-Dawley rats (150 to 250 g) were given Purina laboratory chow, and 28 rats (nine pairs of males and five pairs of females) were pair-fed nutritionally adequate liquid diets (containing 18 percent of the total calories as protein and 35 percent as fat) (*7*). One rat from each pair was given a diet containing 36 percent of the total calories as ethanol, whereas its littermate was fed the control diet in which ethanol had been isocalorically replaced by carbohydrate. After 24 days, the animals were killed; the liver and, in some animals, kidneys, stomach, heart, and brain were quickly excised.

The tissues were homogenized in 1.15 percent KCl, and the subcellular fractions were obtained by ultracentrifugation. The ADH activity was measured (*8*), and it was confirmed that ADH is limited to the cytosol. The following incubation technique was used to study ethanol-oxidizing activity in the microsomes. Microsomes (corresponding to 250 mg of tissue) were incubated at 37°C in a medium containing (per ml) 0.3 μmole of nicotinamide-adenine dinucleotide phosphate (NADP), 5 μmole of magnesium chloride, 20 μmole of nicotinamide, 8 μmole of sodium isocitrate, 2 mg of isocitrate dehydrogenase (crude-type I; Sigma Chemical Co.), 50 μmole of ethanol, and 80 μmole of phosphate buffer (*p*H 7.4). The incubations were carried out in the main chambers of stoppered 50 ml erlenmeyer flasks, with center wells containing 0.6 ml of 0.015*M* semicarbazide hydrochloride in 0.16*M* potassium phosphate buffer (*p*H 7.0). The reaction was stopped with 0.5 ml of 70 percent trichloroacetic acid, and, after an overnight diffusion period at room temperature, the concentration of acetaldehyde bound to the semicarbazide was determined (*9*). That the compound produced by the microsomes was identical with acetaldehyde was shown in two ways: (i) the retention times measured by gas-liquid chromatography (*10*) were equal; (ii) the absorption spectrum of an acetaldehyde-semicarbazone solution was the same as the spectrum of the compound obtained after incubation of microsomes with ethanol. For each determination, at least six incubation flasks were used, with duplicate incubations for 0, 5, and 10 minutes, to verify linearity of the reaction. Activity of the microsomal ethanol-oxidizing system (MEOS) was expressed in units corresponding to the number of nanomoles of acetaldehyde produced per minute per milligram of microsomal protein (*11*).

All hepatic microsomal preparations showed significant ethanol-oxidizing activity that was proportional to the quantity of microsomal suspension. In the ten male rats fed the Purina chow diet, MEOS activity averaged 8.6 ± 0.72 units. The cytosol, mitochondria, and nuclei of the liver, the microsomes of kidney, stomach, heart, and brain as well as purified liver alcohol dehydrogenase (purchased from Calbiochem, New York) had negligible ethanol-oxidizing activity under the conditions of our experiment.