92. Depression of Slow Inward Current by Acetylcholine in Mammalian Ventricular Muscle

By Rikuo OCHI and Naoki HINO

Department of Physiology, School of Medicine,
Juntendo University, Hongo, Tokyo

(Communicated by Yasuji KATSUKI, M. J. A., Oct. 12, 1978)

It is widely known that in cardiac muscle the slow inward current carried mainly by Ca^{2+} is a primary cause for the formation of plateau of action potential and plays an important role in the supply of Ca^{2+} to the contractile machinery. This inward current is depressed by vagal transmitter, acetylcholine (ACh) in the atrial muscle fibers.¹⁾⁻³⁾ With the ventricular fibers, on the other hand, it is traditionally accepted that ACh has little or no effect on the electrical properties. However, ACh depresses the ventricular contractility.⁴⁾ As it has been recently demonstrated that the guinea-pig papillary muscle is densely innervated with cholinergic nerves,⁵⁾ the ventricular muscle of other species could be also innervated in a similar way.

Papillary muscle from the right ventricle of guinea-pig (200–300 g) was excised and mounted on a single sucrose gap chamber. Voltage-clamp was performed applying the current through the gap and recording the membrane potential intracellularly with a glass micro-electrode.⁶⁾ The muscle in the potential pool was so short as both the diameter and length were about 300 μm in order to obtain sufficient space clamp. The potential pool was perfused with normal Tyrode solution*) or solutions containing various concentrations of ACh, the current pool with high potassium solution and the gap between them with isoosmolar sucrose solution. Temperature of all solutions was maintained at about 30°C.

The slow inward current was elicited by depolarizing the membrane to various potential levels from the holding potential of about -40 mV which inactivated the rapid sodium current. One of the results of the action of ACh on the slow inward current is shown in Fig. 1. After the control recording of the membrane current (Fig. 1A), a low concentration of ACh ($5 \times 10^{-7}\text{ M}$) was applied. This caused a slight reduction of the slow inward current (Fig. 1B). Fig. 1 also shows that the ACh effect is dose-dependent. Increasing the concentration to $2 \times 10^{-6}\text{ M}$ caused a further decrease in the slow inward

*) Normal Tyrode solution in mM: NaCl 142, KCl 2.7, CaCl_2 1.8, MgCl_2 1, Tris-HCl 10, glucose 5; pH 7.3.

Fig. 1. Effects of ACh on the slow inward current of guinea-pig papillary muscle. The currents, when the membrane was depolarized for 70 msec from the holding potential of -43 mV to -10 mV (top traces), -1 mV (middle) and $+27$ mV (bottom), are shown. A, in normal Tyrode; B, in 5×10^{-7} M ACh; C, in 2×10^{-6} M ACh; D, in normal Tyrode. Experiment was performed from the left to the right.

current (Fig. 1C). Thereafter, when normal Tyrode was readmitted, the slow inward current was increased reversibly (Fig. 1D). In this experiment the slow inward current was maximal when the membrane was clamped to -1 mV and decreased markedly at $+27$ mV which was slightly negative to the reversal potential of the slow inward current. Neither the time course of the slow inward current nor the reversal potential of the slow inward current appeared to be affected

Fig. 2. Effect of ACh on the current-voltage relationship. The amplitude of the slow inward current was plotted against the clamped membrane potential. (●) and (▲): The peak inward current was measured; holding potential, -35 mV (right arrow); duration of clamp pulse, 80 msec. (○) and (△): The current was measured at the end of the clamp pulse; holding potential, -76 mV; duration of clamp pulse, 800 msec. (●) and (○), in normal Tyrode; (▲) and (△), in 2×10^{-6} M ACh solution. The inserted figures a-d are specimen records obtained at the points a-d on the curves, respectively.

by ACh in spite of the decrease of the peak inward current.

In the mammalian atrial muscle ACh augments the time-independent potassium current at the concentrations lower than those necessary to depress the slow inward current.¹⁾ Fig. 2 shows another example that ACh (2×10^{-6} M) depressed the slow inward current as is given by the specimen records (a and b) and by the plot of the peak inward current against the clamped membrane potential. In order to obtain the action of ACh on the outward current, long (800 msec) clamp pulses were applied from the resting potential. It is clear that neither the inward current during the hyperpolarizing clamp pulses (Fig. 2, c and d) nor the N-shaped current-voltage relationship was affected by ACh. At strong depolarizations to above -20 mV the time-dependent outward current was activated, but this outward current was not enhanced by ACh as evidenced in the plot in Fig. 2. Thus, it can be said that 2×10^{-6} M ACh depresses the slow inward current but does not change much outward current in mammalian ventricular muscle.

Fig. 3. Effect of ACh on the action potential of guinea-pig papillary muscle. A, in normal Tyrode; B, in 5×10^{-7} M ACh; C, in 2×10^{-4} M ACh. Experiments were performed from the left to the right. The preparation was stimulated with long pause of several minutes.

ACh at the concentrations as high as it depressed the slow inward current also depressed the plateau and overshoot of the ventricular action potential as shown in Fig. 3. In spite of this depression, the resting potential, the rapid rising phase and the final repolarizing phase of action potential were not affected appreciably (Fig. 3).

The above results reveals clearly that ACh inhibits the slow inward current at the concentration above 5×10^{-7} M in guinea-pig papillary muscle. Correspondingly, it depresses the plateau of the action potential. This depression of the slow inward current by ACh may play an important role for the negative inotropic effect of vagal stimulation. As it has been established that the slow inward current is augmented by the sympathetic transmitter, noradrenaline and adrenaline, in mammalian ventricular muscle,⁷⁾ the present result suggests that parasympathetic and sympathetic nerves is working in

a reciprocal manner through Ca^{2+} channels in ventricular muscle membranes. As ACh is a natural transmitter, ACh must be a useful tool to separate the slow inward current from other membrane currents in mammalian ventricular muscle.

We thank Prof. H. Mashima for reading the manuscript. The work was supported by the grant from the Japanese Ministry of Education and The Naito Research Grant for 1978.

References

- 1) Ten Eick, R., Nawrath, H., McDonald, T. F., and Trautwein, W. (1976): *Pflügers Arch.*, **361**, 267.
- 2) Giles, W., and Noble, J. S. (1976): *J. Physiol. (Lond.)*, **261**, 103.
- 3) Ikemoto, Y., and Goto, M. (1977): *J. Mol. Cell. Cardiol.*, **9**, 313.
- 4) Watanabe, A. M., and Besch, H. R. (1975): *Circulat. Res.*, **37**, 309.
- 5) Schmid, P. G., Greif, B. J., Lund, D. D., and Koskosk, R. Jr. (1978): *Ibid.*, **42**, 657.
- 6) Ochi, R. (1970): *Pflügers Arch.*, **316**, 81.
- 7) Reuter, H., and Scholz, H. (1977): *J. Physiol. (Lond.)*, **264**, 49.