Evidence of a subsidiary atrial pacemaker in the crista terminalis of the rabbit heart

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Summary. The crista terminalis (CT) with musculi pectinati was isolated from the right atrium: it discharged at a frequency intermediate to that of the 2 nodes. Pacemaker action potentials were recorded from the CT deep layer fibers. The results suggest the presence of a subsidiary atrial pacemaker in the CT deep layer.

Previous research has demonstrated that the crista terminalis (CT) is composed of 2 layers of fibers[1,2]. The deeper fibers differ from the common atrial fibers of the CT superficial layer and musculi pectinati, in that they are clear, large-sized, arranged in parallel and possess T-tubules. They form a homogeneous thick layer which extends to the epicardium for the whole length of the crista. Action potentials recorded from the deep layer fibers have a plateau in the repolarizing phase and a very high maximum rate of rise (V_{max}) and amplitude. These findings led us to attribute to the CT deep layer the role of rapidly conducting excitation to the atrioventricular node (AVN)[2].

Because we occasionally recorded action potentials with a slow diastolic depolarization from the CT deep layer and noting, in addition, that the presence of atrial ectopic foci have been localized by extracellular recordings in the dog's heart[3-5], experiments were carried out to determine whether the CT deep layer fibers can act as pacemaker when separated from the sinoatrial node (SAN) and, eventually, from the AVN.

Methods. Hearts were excised from 30 rabbits (weighing 2.5-3 kg) anesthetized with pentobarbitone sodium. The isolated right atrium was prepared as described by Paes de Carvalho et al.[6] and subsequently dissected into 3 preparations: 1. the SAN, 2. the CT and musculi pectinati, 3. the AVN. The 3 preparations, maintained at 37 ± 0.3 °C, were perfused with a Tyrode solution, equilibrated with O_2 95% and CO_2 5%. The composition of the solution in mM was: NaCl 137, KCl 2.7, $CaCl_2$ 1.9, $NaHCO_3$ 12, glucose 5.5, $MgCl_2$ 0.5, NaH_2PO_4 3.6. The pH of the solution was between 7.2 and 7.4. Further dissection to expose the deep layer of the CT was done carefully by peeling off a thin strip of the superficial layer. Action potentials and V_{max} were recorded from the fibers of the 3 preparations with floating glass microelectrodes by conventional methods.

In order to verify that the CT was properly isolated and that the tissue was well preserved, microscopic and ultrastructural controls were made. The tissue was fixed by substituting a fixing solution for the Tyrode solution, either a Karnowsky diluted solution[7] or the fixing solution referred to by Janse et al.[8].

Results and discussion. Following the dissection of the SAN region from the beating right atrium the isolated SAN continued discharging at a mean rate of 156 ± 7/min. The remaining part, after a few seconds' standstill, started to discharge at a mean frequency of 126 ± 10/min. Dissection of the AVN from the CT preparation caused standstill of the former, while the CT continued discharging at the same

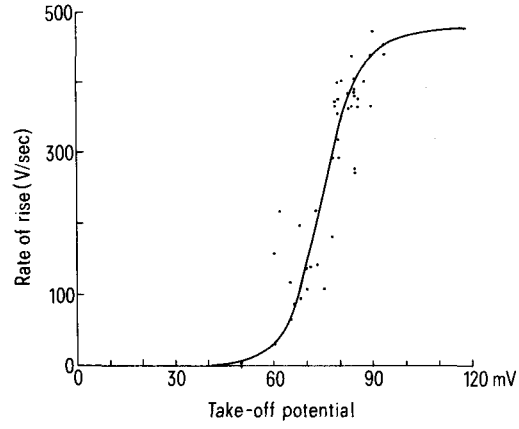

Figure 2. Relationship between take-off potential and maximum rate of rise of action potentials recorded from the isolated CT deep layer fibers. The sigmoidal curve was drawn from equation (1).

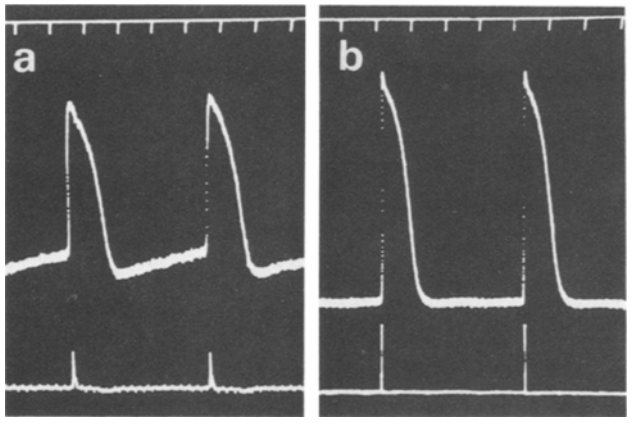

Figure 1. Action potentials and maximum rates of rise recorded from the deep layer of the crista terminalis: *a* from the pacemaker region, *b* from the normal CT deep layer fibers. Amplitude calibration is 100 mV, V_{max} calibration is 100 V/sec in (a) and 200 V/sec in (b). Time: 100 msec.

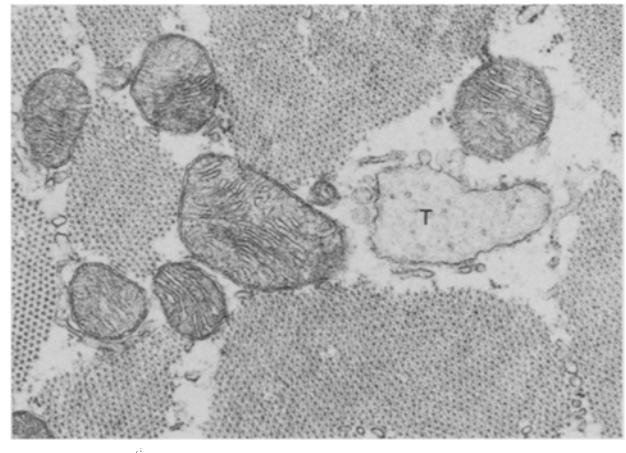

Figure 3. Electron micrograph of a fiber from the deep layer of the crista. The well-preserved state of the tissue is apparent from the fine morphology of mitochondria intercalated between myofibrils and undifferentiated sarcoplasm. Moreover a large transverse tubule (T) with micropinocytotic vesicles and profiles of sarcoplasmic reticulum are observable. × 27.800.

rate. The mean rate of discharge of the isolated AVN was 72 ± 11/min.

10 CT preparations, when isolated from the musculi pectinati, continued discharging. These were subsequently cut transversely into 2–4 pieces: after variable times (up to 1 min) each discharged at a rate varying between the initial rate of the entire CT and its half value. In 3 of the preparations, 1 CT piece did not show automaticity.

Action potentials with a variable slope of diastolic depolarization were recorded from a limited area of the deep layer of any spontaneously discharging isolated CT-musculi pectinati preparation, as shown in figure 1a. The amplitude of these action potentials was between 80 and 90 mV and their V_{max} between 90 and 140 V/sec. The location of the pacemaker area varied within the CT deep layer. Similar pacemaker potentials were recorded by Paes de Carvalho et al.[6] from the sinoatrial ring bundle, but not from the superficial CT layer. An example typical of action potentials recorded from the deep layer outside the pacemaker region is shown in figure 1b. These action potentials were homogeneous, had a mean amplitude of 114 ± 2.1 mV and a mean V_{max} of 374 ± 14 V/sec.

V_{max} of action potentials were plotted against the take-off potentials of all records of the CT deep layer and a sigmoidal relationship was found (fig. 2). Experimental data are in good agreement with the sigmoidal curve drawn from the equation of Hodgkin and Huxley[9]

$$h = \frac{1}{1 + \exp(V_h - V)/s} \qquad (1)$$

where, according to Weidmann[10], h is the fraction of the highest value observed for the rate of rise, V the take-off potential in mV, V_h the potential at which h is half maximum and s the slope factor. $V_h = 75.3$ mV and $s = 5.78$ were determined according to Noma and Irisawa[11]. The sigmoidal relationship found is similar to that reported by Weidmann[10] in the ventricular Purkinje fibers but largely differs from that in sinoatrial pacemaker fibers[11]. Another resemblance to Purkinje fibers is the relatively steep rate of rise.

The fine morphology and particularly the ultrastructural features of the mitochondria shown in figure 3, demonstrate the excellent in depth preservation of the CT, comparable to the state of preservation of the CT excised from hearts perfused by the Langendorff method[1].

The present findings demonstrate that a) the CT deep layer fibers can act as pacemaker, discharging at a rate intermediate to the rates of the 2 nodes. b) Pacemaker activity, as a property of the deep layer cells, can be observed throughout the CT (all the pieces cut from it discharged spontaneously). c) The high discharge rate recorded from the isolated crista indicates that this area, being inherently faster than the AVN pacemaker, would probably be the faster pacemaker in the absence of SAN pacing. d) From the electrophysiological characteristics a resemblance of the CT deep layer pacemaker potentials and those of Purkinje fibers emerges.

1 G.L. Avanzino, D. Blanchi, A. Calligaro, R. Ermirio and M. Fisher, J. Physiol. *313*, 10 (1981).
2 G.L. Avanzino, D. Blanchi, A. Calligaro, R. Ermirio and M. Fisher, J. Physiol. *313*, 81 (1981).
3 W.C. Randall, J. Talano, M.P. Kaye, D. Euler, S. Jones and G. Brynjolfsson, Am. J. Physiol.; Heart Circ. Physiol. *3*, H465 (1978).
4 S.B. Jones, D.E. Euler, W.C. Randall, G. Brynjolfsson and E.L. Hardie, Am. J. Physiol.; Heart Circ. Physiol. *7*, H788 (1980).
5 J.P. Boineau, R.B. Schuessler, D.B. Hackel, C.B. Miller, C.W. Brockus and C. Wylds, Am. J. Physiol.; Heart Circ. Physiol. *8*, H406 (1980).
6 A. Paes De Carvalho, W.C. de Mello and B.F. Hoffman, Am. J. Physiol. *196*, 483 (1959).
7 M.J. Karnovsky, J. Cell Biol. *27*, 137A (1965).
8 M.J. Janse, J. Tranum-Jensen, A.G. Kleber and F.J.L. van Capelle, in: The sinus node, p. 183. Ed. F.I.M. Bonke. Martinus Nijhoff Medical Division, The Hague 1978.
9 A.L. Hodgkin and A.F. Huxley, J. Physiol. *116*, 497 (1952).
10 S. Weidmann, J. Physiol. *127*, 213 (1955).
11 A. Noma and H. Irisawa, Jap. J. Physiol. *24*, 617 (1974).

Metabolism-weight relationship in 17 humming-bird species at different temperatures during day and night

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Summary. The mean metabolic rate during day and night of 17 different humming-bird species is considerably higher than the expected value for nonpasserine birds. The weight-metabolism regression exponent for the night-time is in the same range as that reported for other avian orders (and mammals); 0.73.

Previous studies have established a higher basal metabolism in the Passeriformes in comparison to the other avian orders (Nonpasseres). Our investigations in 24 different relatively small nonpasserine birds[1], however, showed no pronounced differences relating to the metabolic rate per unit body mass. As a comprehensive analysis of the thermoregulatory process requires adequate knowlegde of the levels of energy production, it was of great interest to study humming-birds, which include the smallest (nonpasserine) bird species.

Materials and methods. The humming-bird species examined are listed in the table. The metabolism of each species was continuously recorded during a period of at least 5 consecutive days (and nights) at different environmental temperatures between 2 °C and 25 °C. Each temperature was tested for 24 h (1 day). The dark-light cycle was 12:12 h; food was provided ad libitum. Measuring instruments: Hartmann & Braun Magnos 2T and Uras 2T (6 channels). For more details see Prinzinger[2].

Results and discussion. Both during day and night, and at all temperatures tested the metabolism of all the humming-birds was considerably higher than the theoretically expected value for nonpasserine birds (fig.). This fact could also be confirmed for nights, in which torpor (strongly reduced metabolic rate) occurred. The mean metabolism-weight regression line of the day-time values follows the equation $M = 0.83 \cdot W^{0.56}$ (M = metabolism in KJ/h and W = b.wt in g). That of the night-time values is $M = 0.67 \cdot W^{0.73}$. The regression exponent of the night-time values corresponds satisfactorily with the results of previous examinations (Dawson and Hudson[3], 0.720; Aschoff and Pohl[4], 0.734; Prinzinger and Hänssler[1], 0.716). During the day-time the