The site of gating in the ventricular conducting system of rabbit, dog and monkey hearts

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Summary. The longest action potential durations of the ventricular conducting system were found at about two thirds of the distance along each false tendon in dog and monkey hearts. In the rabbit heart, this area - which corresponds to the gating mechanism - was found in the middle part of the bundle branches; therefore the number of gates is much smaller in the rabbit than in the other 2 species.

Experiments performed on dog hearts have shown that the action potential duration (APD) increases progressively along the course of the conducting system distally from the bundle of His[2]. APD reaches a maximum value at a distance of 2-3 mm from the Purkinje-ventricular myocardium junction[3], then decreases from the distal Purkinje fibers to transitional and muscle cells[4]. The area of fiber with the greatest APD acts as a gate which prevents the propagation of premature impulses across the distal conducting system[5,6], and determines the functional refractory period of the system. From this point of view, the main role of the gating mechanism seems to be to protect the heart against ventricular fibrillation.

Our present study was undertaken to compare APD along the conducting system in 3 different species of mammals, namely the dog, monkey and rabbit. We observed that the location of the gate was different in the rabbit from that in the dog and monkey.

Methods. Mongrel dogs were anaesthetized with sodium pentobarbital at a dose of 30 mg/kg i.v., and monkeys with phencyclidine chlorhydrate at a dose of 3 mg/kg i.v. Rabbits were killed by a blow on the back of the neck.

In the case of adult dogs, the bundle branch, papillary muscle, free running false tendons and a small piece of the free wall of the right and left ventricle were dissected out after carefully opening the ventricle. In the case of rabbits, monkeys and puppies, because of the rather small size of the hearts and/or fine and multiple ramifications of the Purkinje network, measurements of APD were performed in the open right or left ventricle; it has been previously demonstrated that survival of Purkinje and subendocardial muscle fibers is satisfactory using this preparation[7,8]. However, a few experiments were performed on isolated rabbit bundle branch-Purkinje fiber-myocardium preparations, great care being taken to avoid stretching of the false tendons which results in an anomalous lengthening of the action potential plateau and a decrease in its amplitude. All preparations were allowed to recover for at least 45 min after dissection.

Tyrode's solution had the following composition (mM): NaCl: 137; $NaHCO_3$: 12; $CaCl_2$: 2.7; KCl: 5.4; $MgCl_2$: 1.05; NaH_2PO_4: 1.8; dextrose: 11.0; pH: 7.4 (saturated with O_2 95%; CO_2 5%). Temperature within the bath was maintained at 37 ± 0.5 °C. Standard microelectrodes mounted in a semi-flexible way were used. Preparations were stimulated through a bipolar extracellular electrode insulated with teflon, except at its tip, and generally positioned in the upper part of the bundle branch (the cycle length was 800 ms). The recording microelectrode was moved in 1-3 mm steps (according to the size of the heart) from the proximal right bundle branch along a false tendon up to the myocardium. At each position, action potentials were recorded and APD measured (at 80% repolarization). Though the preparations were very stable, all the action potentials were generally recorded in less than 15 min for a given set of measurements. In 6 rabbit hearts, APD was measured from the lower part of the bundle of His.

Results. 1. Dog: Our results fully confirmed the distal localization of the gate (figure 2, A and D). However, in adult dogs, the greatest APD was generally found 4-6 mm proximal to the termination of conducting fibers in the free ventricular wall, in agreement with Wittig et al.[6] rather than 2-3 mm, as measured by Myerburg et al.[9]. We also observed that the localization of the gate is very similar in right and left ventricles and also in isolated preparations (adult dogs) and in situ preparations (puppies). It can be seen in figure 2, A that although APDs differ from heart to heart, the shapes of the curves are very similar in the different preparations.

2. Monkey: The size of the heart (linear dimensions) was about twice as small in our monkeys than in our adult dogs. The Purkinje network was much more ramified and the bundles were much thinner. In spite of these morphological differences, the APD and the localization of the gate were found to be very similar in the monkey (figures 1, 2, B and D) and in the dog, though in the former species the area of maximal APD was somewhat less distal than in the latter.

3. Rabbit: The Purkinje network of the rabbit heart resembles that of the monkey, with even thinner bundles. As previously described[9], the shape of the Purkinje fiber action

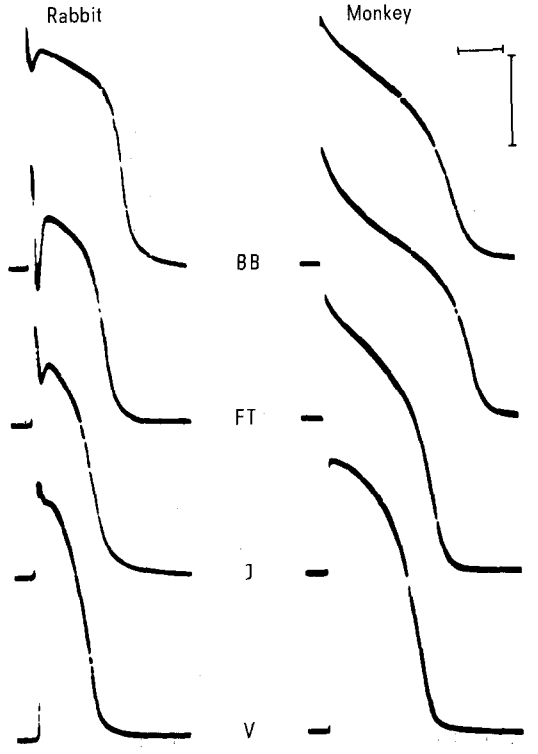

Fig. 1. Action potentials recorded at different sites along the conducting system of the isolated superfused left ventricle in the rabbit (left column) and the monkey (right column). BB: bundle branch; FT: false tendon; J: Purkinje-myocardium junction; V: ventricle. Calibrations: 100 msec and 40 mV.

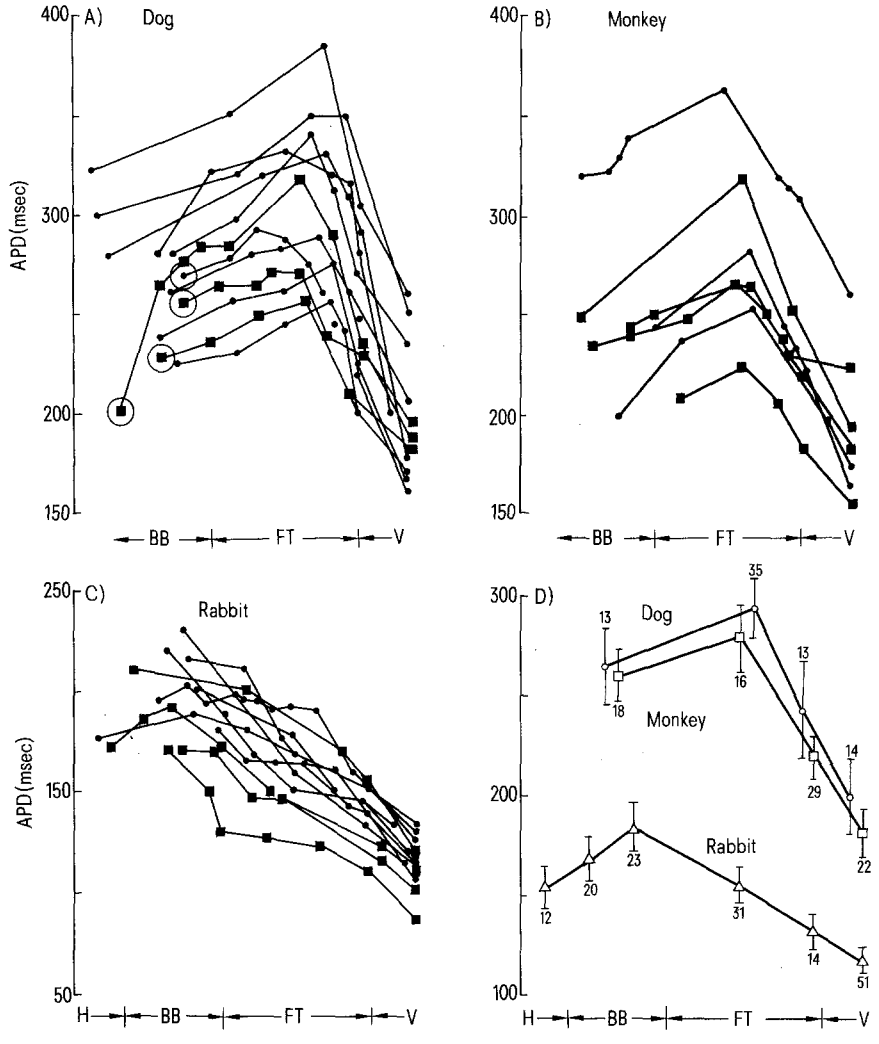

Fig. 2. Action potential duration (APD) recorded at different sites along the conducting system in the dog (A), monkey (B), and rabbit (C). H: bundle of His; BB: bundle branch; FT: false tendon and V: ventricle. ●: right ventricles, ■: left ventricles. ◉ and ◉: data for puppies. D: mean values of ADP in the dog, monkey and rabbit from experiments shown in A, B and C and from other measurements corresponding to partial exploration of the conducting system. Vertical bars: ± confidence intervals (numbers of impaled fibers given). Horizontal scale: the position of dots along the abscissa have been calculated by giving to each false tendon a length equal to unity. The false tendon lengths varied as follows: in adult dogs: 15–25 mm (right ventricles, RV) and 13–20 mm (left ventricle, LV); in monkeys: 8–13 mm (RV) and 5–11 mm (LV); in puppies and rabbits: 8–11 mm (RV) and 5–10 mm (LV).

potential in the rabbit heart showed a marked dip between phases 1 and 2, in contrast to what occurs in monkey (figure 1) and dog Purkinje fibers. Another difference concerned the localization of the gate; the greatest APD occurred not in the false tendons but approximately half-way along the right and left bundle branches (figures 1, 2, C and D). The absence of measurements in the proximal part of the right bundle branch (figure 2C) is due to the fact that this part of the branch is impossible to impale because it lies deep in the septum. To ascertain that the area of maximal APD lies in the bundle branch, we recorded the AP from the bundle of His in several rabbits as shown in figure 2, C and D.

To test whether some shortening of the Purkinje fiber action potential might occur as a consequence of multiple Purkinje fiber muscle junction[11], we cut the lateral short branches leaving a main false tendon. These transections brought no change in the APD distribution (3 experiments). Similar results were obtained in 5 isolated bundle branch – Purkinje fiber-myocardium preparations.

In 2 experiments we compared the durations of action potential as recorded from a) the site of maximal APD and from b) the middle part of a false tendon. Increasing the driving rate from 1/sec to 6.6/sec shortened both action potentials but did not alter the ratio of their durations to any significant extent. This suggests that in the rabbit heart the gate remains more effective at high frequencies than in the dog heart[9].

Discussion. The fact that the longest conducting system action potentials have been found in the distal part of the Purkinje fibers in dog and monkey hearts and in the middle part of the bundle branch in rabbit heart might be related to the different sizes of the hearts. This, however, is not the case, since in our experiments the sizes of the monkey, rabbit and puppies' hearts were comparable. The morphology of the Purkinje network is similar in the rabbit and monkey and rather different in the dog. Our results concerning the rabbit heart are at variance with those reported by Salako et al.[12]. These authors tate that in isolated right bundle branch-Purkinje fiber-muscle preparations the maximal APD was found in the false tendons, and reached a value of about 300 msec at 35 °C, whereas in our experiments the greatest APD found in false tendons was about 200 msec at 37 °C. Recent experiments have shown that in the dog heart a shortening of Purkinje fiber APD can result from the proximity of the low ohmic resistance Purkinje fiber-muscle junctions[11]. Since in the rabbit heart false tendon ramifications are often somewhat shorter than in other species, it might be argued that Purkinje fiber-muscle junctions exert a repolarizing influence up to a part of the false tendon proportionally longer than in other species. However our observation that cutting all the lateral branches of a false tendon does not change the distribution of APDs along this false tendon is contrary to the hypothesis of electronic interaction.

The reason for the discrepancy between our results and those of Salako et al.[12] remains unexplained. It may be related to differences in the ages of the animals, since it has been observed that in the monkey heart the shape and

duration of the Purkinje fiber action potentials vary noticeably with age (Walden and Kreher, personal communication).

According to Myerburg et al.[5], the fact that a gating mechanism is located in every false tendon may be a source of arrhythmias since alteration of only 1 of these gates can trigger re-entry. The probability that 1 of the gates is altered becomes obviously greater when the number of gates increases. From this point of view, the rabbit heart would be less exposed to certain types of arrhythmias than the dog or monkey hearts since the number of gates is limited to 3, namely the 3 bundle branches (1 in the right ventricle, and 2 in the left ventricle).

The fact that the localization of the gate is almost identical in dog and monkey and is somewhat different in rabbit is a new observation. This result is rather suprising in view of the very different morphological structures of the conducting systems in dogs and monkeys and their similarities in monkeys and rabbits.

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2 B.F. Hoffman, E.N. Moore, J.H. Stuckey and P.F. Cranefield, Circulation Res. *13*, 308 (1963).
3 R.J. Myerburg, J.W. Stewart and B.F. Hoffman, Circulation Res. *26*, 361 (1970).
4 C. Mendez, W.J. Mueller, J. Merideth and G.K. Moe, Circulation Res. *24*, 361 (1969).
5 R.J. Myerburg, H. Gelband and B.F. Hoffman, Cardiovasc. Res. *7*, 69 (1973).
6 J. Wittig, L.A. Harrison, A.G. Wallace and N.C. Durham, Am. Heart J. *86*, 69 (1973).
7 Sz. Viragh, P. Gautier, E. Coraboeuf and A. Porte, J. Microscopie, Paris, *20*, 98A (1974).
8 P. Gautier, thesis, University of Orsay 1973.
9 R.J. Myerburg, H. Gelband and B.F. Hoffman. Circulation Res. *28*, 136 (1971).
10 E.A. Johnson, and J. Tille, J. gen. Physiol. *44*, 443, (1961).
11 J.C. Bailey, D.A. Lathrop and D.L. Pippenger, Circulation Res. *40*, 464 (1977).
12 L.A. Salako, E.M. Vaughan Williams and J.H. Wittig Br. J. Pharmac. *57*, 251 (1976).

Effect of glycerol treatment on sodium and potassium in isolated muscle fibres of the frog

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Summary. The sodium concentration in single frog muscle fibres vacuolated by glycerol treatment was significantly higher than in devacuolated fibres. Intracellular potassium concentration did not show any significant change. It is concluded that the transverse tubular system forms vacuoles with a high NaCl concentration upon glycerol removal.

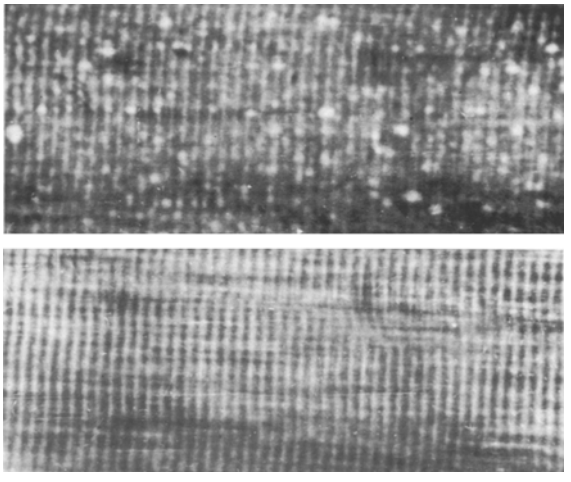
Muscle fibres, preincubated for 30 min in Ringer made hypertonic by the addition of 220 mM/l of glycerol, exhibit vacuolation of the transverse tubular system (TTS) after being exposed to normal Ringer[1,2]. Reapplication of glycerol-Ringer to vacuolated fibres results in the disappearance of vacuoles. These processes play an essential role in the decoupling and recoupling of the excitation-contraction link in muscle fibres. Recently it was shown that a TTS vacuolated by hypertonicity[3] and fatigue[4] has a high NaCl concentration. The ion content in vacuoles caused by glycerol removal is not yet clear. The purpose of this study was to compare the sodium and potassium concentrations in fibres vacuolated by glycerol removal with those in fibres devacuolated by reapplication of glycerol.

Experiments were performed on single muscle fibres isolated from the m.iliofibularis of the frog *(Rana temporaria)*. The following solutions were used: a) glycerol-Ringer (solution 1): concentration in mmole/l: NaCl 111.0, KCl 2.4, $NaHCO_3$ 2.4, $CaCl_2$ 1.8, glycerol 220.0; b) Ringer (solution 2): the same composition as for solution 1 plus a constant amount of 3.2 mmoles/l $CaCl_2$ and $MgCl_2$ without glycerol. All fibres were equilibrated for 30 min in solution 1 and after that for 30 min in solution 2. Light microscopy of these fibres revealed the presence of vacuoles distributed

The effects of glycerol removal and reapplication on sodium and potassium in skeletal muscle fibre of the frog

Group	Treatment	$[NA^+]$ mmole/kg fibre water	$[K^+]$ mmole/kg fibre water
1	Solution 1 for 30 min followed by solution 2 for 30 min	45.8±7.3 n=9	125.1±8.6 n=9
		p<0.002	p>0.1
2	The same as for group 1 followed by solution 1 for 30 min	21.8±2.0 n=15	124.8±6.8 n=14
		p>0.1	
3	Solution 2 for 120 min	25.6±2.6 n=13	
		p>0.1	
4	Solution 1 for 120 min	23.1±1.4 n=9	

Data are means±SEM. n, number of determinations; p, values were determined using Student's t-test (dispersion of $[Na^+]$ in group 1 as compared to groups 2, 3 and 4 was different). There were significant differences between $[Na^+]$ in group 3 ($p < 0.05$) and 4 ($p < 0.02$) when compared to group 1.



The light microscopic structure of frog muscle fibre ×400. *a* The vacuolated structure after washing out the glycerol. *b* The normal fibre after reapplication of glycerol.