Oscillatory after-potentials and triggered-automaticity in mammalian ventricular muscle fibres at high resting potentials

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Summary. Oscillatory after-potentials and triggered-automaticity were observed in dog ventricular muscle fibres when the fibres were exposed to K^+-free,high-Ca^{++}-solutions after K^+-free,Ca^{++}-free perfusion. They appeared at membrane potentials more negative than −60 mV.

Automaticity has been considered as a characteristic feature of the specialized tissues of the heart, and it is generally believed that the working myocardium lacks automatic activity under physiological conditions[1]. However, recently several reports presented evidence of abnormal automaticity in the working myocardium under certain conditions[2–6]. Most of these cases, however, showed depolarization of resting potentials of about −50 mV or less when myocardium developed automaticity. Thus the critical question may arise whether the working myocardium does show automaticity at resting potentials more negative than −60 mV. To approach this critical question, we examined the effects of K^+-free solution either with low or high Ca^{++}-concentrations on dog ventricular muscle fibres, since K^+-free,Ca^{++}-free condition was shown to induce automaticity in the working myocardium[6].

Material and methods. Fine papillary or trabecular muscles which appeared not to contain Purkinje fibres were dissected from the right ventricle of dogs removed under sodium pentobarbital anesthesia. Preparations were placed in the tissue chamber where modified Tyrode solution was perfused. The modified Tyrode solution had the following composition (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, glucose 5.5, NaH_2PO_4, 0.42, $NaHCO_3$ 11.9. Sometimes, 10 mM Tris buffer instead of bicarbonate buffer was used. K^+-free and K^+-free,Ca^{++}-free solutions were made up by omitting KCl and $CaCl_2$ from the modified Tyrode solution. K^+-free,high-Ca^{++} solutions were prepared by adding extra-$CaCl_2$ in K^+-free solution. Solutions made by bicarbonate buffer were bubbled with 95% O_2+5% CO_2 and those by Tris buffer with 100% O_2. Temperature of the perfusate was maintained at 36–37 °C. The conventional microelectrode technique was employed to measure membrane potentials.

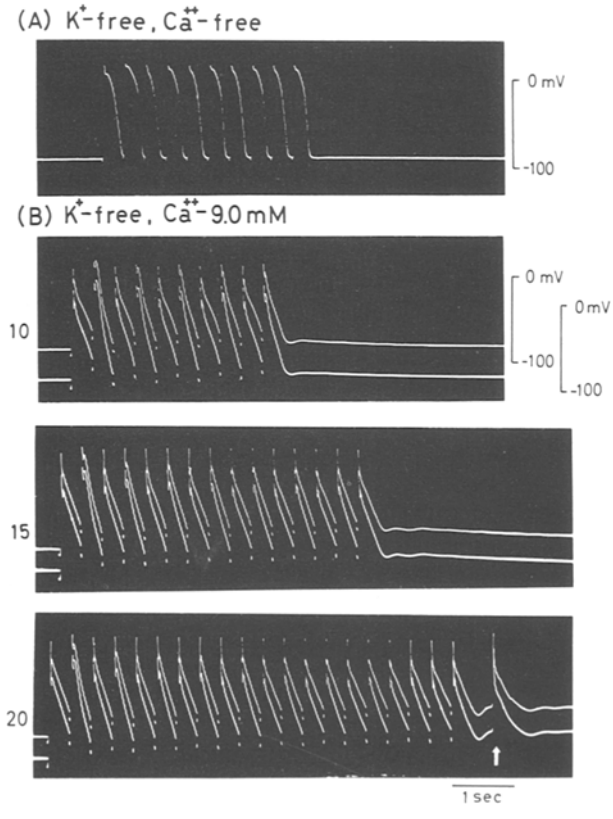

Fig. 1. Oscillatory after-potentials and extrasystole induced in K^+-free,high-Ca^{++}-solution. In (A), the record was taken in K^+-free,Ca^{++}-free solution. No after-potentials were seen after the train of action potentials. (B) shows the records taken in K^+-free,9.0 mM-Ca^{++}-solution with the same preparation as in (A). 2 records in each picture were taken simultaneously from the 2 different cells in the same preparation. The inter-electrode distance was 5.25 mm. The numbers at the left of each picture indicate numbers of the train of impulses. OAPs of low amplitude developed following the last driven action potentials. The amplitude of OAPs became larger with increasing the numbers of the train of impulses. When the train of 20 impulses was applied, an extra-excitation appeared as indicated by the white arrow in the bottom record. Basic cycle length of the train was 320 msec in all the records.

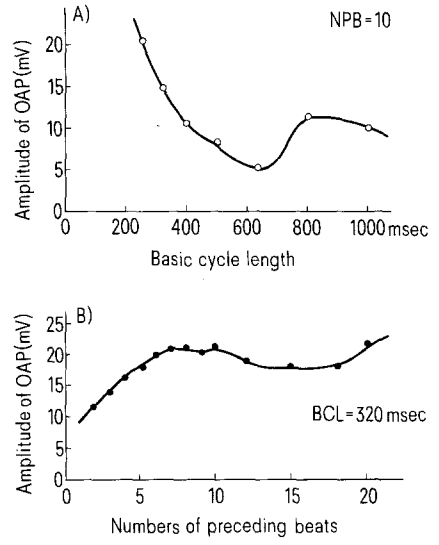

Fig. 2. Dependence of OAPs on basic cycle length (A) and on the numbers (B) of the train of impulses. The amplitude of OAP was measured by the difference between the maximum and the minimum point of OAP. In (A), the shorter the basic cycle length, the larger the amplitude of OAPs. But they usually showed secondary rise at the basic cycle length between 500 and 800 msec depending on the preparations. Therefore the dependence of OAPs on the basic cycle length exhibited rather a complicated manner than a single smooth one. NPB means the number of the preceding beats. In (B), the dependence of OAPs on the number of the preceding beats showed an increase with increasing the numbers of the train of impulses, but it again showed nonlinear rise. The lines in (A) and (B) were drawn by eye.

Results and discussion. Each preparation was initially driven at a basic cycle length of 1000 msec for 30 min. If the preparations were found to show the typical shape of Purkinje action potentials[1], they were discarded. After being stabilized in the modified Tyrode solution, the tissue was perfused with K^+-free solution for 30 min without stimulation. If the preparations developed either spontaneous firings or depolarization of resting potentials to −60 mV or less, they were omitted from the further experiments since they might contain Purkinje fibres. After this period, the tissues were perfused with K^+-free,Ca^{++}-free solution for another 30 min and all the fibres which showed resting potentials more negative than −60 mV never developed spontaneous activity during this perfusion period. At the end of this perfusion, Ca^{++}-concentration in the medium were suddenly increased to 3.6–18 mM. The value of resting potentials at this stage showed −93.5±1.69 mV (mean±SE, n=21). When a train of impulses was applied to the preparation and suddenly stopped, low amplitude oscillatory afterpotentials of 2–5 in numbers appeared following the last driven action potentials, which were never seen in K^+-free,Ca^{++}-free solution (figure 1). These oscillatory after-potentials (OAPs) became larger with increasing the number of the driven action potentials. If they were large enough to reach the threshold, an extra-excitation triggered by the train ensued (figure 1, B, bottom).

This triggered-automaticity appeared not only as a single extrasystole but also more than 2, and occasionally as self-sustaining tachycardias. The average threshold potential of the triggered-automaticity induced in 10 preparations repeatedly was −70.1±3.17 mV (mean±SE, n=10). Once OAPs appeared, they could be recorded with every impalement of 6 to 12 different sites in each preparation. The amplitude of OAPs showed dependence not only on the numbers of the train but also on the basic cycle length (figure 2).

The present results were different from the findings reported by Müller in the working myocardium[6], since he observed automatic activity in K^+-free,Ca^{++}-free solution at decreased resting potentials, and no OAPs nor triggered-activity were described. OAPs and triggered-activity were mainly regarded as a characteristic feature of slow responses or observed in depolarized fibres[7,8], or activities limited to special regions of the heart[9–12]. The present experiments disclosed that ventricular muscle fibres could equally develop OAPs and triggered-automaticity at potentials more negative than −60 mV. Thus these results indicate that OAPs and triggered-automaticity are not unique features of the specialized tissues, but represent a general character inherent to all the cardiac muscles, and appear either at high or low resting potential levels. The mechanism of this OAP is not clear from the present experiments, but we assume that Ca^{++}-movement across the cell membrane under the decreased K^+-conductance may be operative as a basis of this OAP, since it could be induced only in K^+-free,high-Ca^{++} solutions.

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A simple method for observation of capillary nets in rat brain cortex

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Summary. By the authors' technic, the profiles of capillaries in rat brain cortex were clearly demonstrated. The capillaries formed complicated nets by sprouting of their finer branches and anastomosing with each other. Further, a kind of perivascular cells with yellow autofluorescent granules was distributed close to capillaries, arterioles or venules. They seemed to be a special form of macrophage in the brain cortex.

The distribution-pattern of capillaries in brain cortex had been studied with some technics using carbon particles or colouring matters[1]. Recently, some fluorescent substance was also employed for this purpose. However, it was difficult to follow a running course of capillaries by those methods, even if serial sections were employed.

On the other hand, according to de la Torr[2], endothelial cells specifically took up administered L-DOPA and emitted a green fluorescence. Further, by the authors' tentative observation, a kind of pericytes with yellow fluorescent granules was distributed close to capillaries in the cortex. Based on these findings, the authors tried to establish a simple method for a representation of a running course of capillaries and a distribution of fluorescent cells. However, it is difficult to elucidate a distribution of fluorescency emitted from L-DOPA by routine histological procedures, because L-DOPA was easy to diffuse into surrounding tissues.

After several trials, it was known that the following procedure was available for the authors' purpose. At 2 h after the administration of 10 mg of safrazine (monoamine oxidase inhibitor), 10 mg of L-DOPA was injected to Wistar rats weighing 230–250 g, s.c. After 1 h, the rats were anesthetized, and their brains were removed. Then the pieces excised from a cortex were placed on nonfluorescent glass slide and pressed by hand with the other opposite glass slide. And the slide was moved to a right side to get stretched specimens. The specimens were dried with electric fan for 20 min at a room temperature and placed in a desiccator for 20 min at 10^{-3} Torr. They were exposed to formaldehyde gas for 1 h at 80 °C for a detection of L-DOPA by Falck-Hillarp method[3] and examined under a