The influence of hypoxia and metabolic inhibitors on the excitation process in atrioventricular node

Der Einfluß von Hypoxie und Stoffwechselinhibitoren auf den Erregungsprozeß des Atrioventrikularknotens

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With 9 figures

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Summary

In small specimens prepared from the atrioventricular node of rabbits, the influence of hypoxia and metabolic inhibitors (Na-cyanide, 2.4 dinitrophenol, 2-desoxyglucose) on the av-node action potential was studied. The action potentials in these specimens proved to be of slow response type regardless of their origin, either the N or the NH-region of the node.

1. Following superfusion with O₂-poor Tyrode solution, $V_{\text{max}}$ of the atrioventricular action potential gradually declined within 90 min and attained finally a new steady state. A mean decrease of $30.2 \pm 9.4\%$ was obtained. Frequency of automatic impulse formation went down by almost the same amount within only 20 min. On return to an oxygenated Tyrode solution, or in the continued presence of O₂ deficiency after an increase of the external glucose concentration from 11 mM to 33 mM, full recovery of these effects occurred.

2. After administration of Na-cyanide (1 $\times$ 10⁻³ M), a similar $V_{\text{max}}$ decrease of $29.0 \pm 7.0\%$ appeared. It took only 20 min for full development and was accompanied by a decrease of frequency of automatic impulse formation by $47.0 \pm 10.1\%$ which occurred very rapidly within 2–3 min. Both cyanide-induced effects proved reversible within 10–20 min on return to a cyanide-free medium. Poisoning the atrioventricular cell with 2.4 dinitrophenol (1 $\times$ 10⁻³ M) led to the same result. Treatment with 2-desoxyglucose (3 $\times$ 10⁻² M) evoked a more pronounced $V_{\text{max}}$ diminution of 50%.

3. The inhibitor of K conductance, 4-aminopyridine (2 $\times$ 10⁻³ M) did not remove the metabolically induced changes of $V_{\text{max}}$ of the atrioventricular action potential. After poisoning of oxidative phosphorylation, this compound caused in some cases even a further reduction of $V_{\text{max}}$.

4. The β-adrenergic compound isoproterenol (9.2 $\times$ 10⁻⁶ M) restored the hypoxia or cyanide-induced suppression of both $V_{\text{max}}$ and frequency of automatic impulse formation. The particularly pronounced response of $V_{\text{max}}$ led to an increase far exceeding the initial control values obtained under normal metabolic conditions of the atrioventricular cell.

Metabolic interventions are well known for strongly affecting cardiac excitation. As first described by Trautwein et al. (35), in hypoxic ventricular myocardium the immediate response of the cardiac action potential to an impaired cellular ATP synthesis is a significant loss of plateau and a marked shortening whilst upstroke velocity changes occur much later.
simultaneously with a decline of resting potential (for review see Carmeliet (4)). A quite different pattern of reaction will be obtained if excitation is mediated by the slow inward current rather than the fast Na system. The resultant slow response action potential is conspicuous in that it responds with a rapidly occurring and significantly pronounced $V_{\text{max}}$ decrease (31). A similar sensitivity of $V_{\text{max}}$ to hypoxia or metabolic inhibitors has been found in the sinoatrial node (13, 20, 32) where the slow inward conductance system is mainly responsible for depolarization (for review see Irisawa (9)), which, however, is not to be considered as being proof for a direct dependence of this special form of excitation on the metabolic state of the cardiac cell.

In contrast to working myocardium and sinoatrial node, the dependence of excitation process on cellular energy production still remains to be further clarified in the atrioventricular node. One could argue that a decline of ATP synthesis results in changes related to those appearing in the sinoatrial node since the majority of atrioventricular cells very probably generate, as physiological mode of excitation, action potentials of slow response type, too. As first demonstrated by Hoffman et al. (7), the rate of rise is much lower than that of atrial or ventricular action potentials and the upstroke phase is lacking a fast component (23). Stronger arguments in favour of the slow response character are the susceptibility to the slow channel inhibitors, Mn ions (40) and verapamil (38), and the ineffectiveness of TTX, which has proved incapable in depressing atrioventricular action potentials both in N and in NH-cells in concentrations sufficient for blockade of fast Na channels (40). Inconsistent with these observations are recent results of Ruiz-Ceretti et al. (29), which revealed an initial TTX-sensitive fast upstroke component even in nodal action potentials.

In the present study, the shape of the rising phase of the atrioventricular action potential including its susceptibility to promoters and inhibitors of slow inward current, isoproterenol and verapamil, has, therefore, been reinvestigated thereby employing small specimens of the av-node, which offer a successful approach for continuous microelectrode impalements. In the same preparation, the effect of hypoxia and metabolic inhibitors has been analyzed in an attempt to estimate the role of oxidative phosphorylation for excitation in av-node. Finally, it proved of interest to test as to whether energy depletion might alter the typical changes of the atrioventricular action potential induced by $\beta$-adrenergic catecholamines.

**Methods**

Rabbits of either sex (weight 1.5-2.5 kg) were killed by a blow on the neck and the hearts were rapidly removed. In a dissection chamber continuously perfused with oxygenated Tyrode solution (Ca concentration 2 mM) the anterior wall of both right atrium and right ventricle and the free wall of both left atrium and left ventricle were removed. The remaining preparation consisting of septum interventriculare and septum interatriale was stepwise reduced in size until the av-node region including a small residue of both septa of about 4-5 mm in width had been isolated. Using special razor blades or a precision scissor, the atrial tissue containing the av-node was carefully isolated from the septum interventriculare, thereby avoiding any stretching of the preparation. The majority of the av-node prepara-