The Blockade of $V_{\text{max}}$ of the Atrioventricular Action Potential Produced by the Slow Channel Inhibitors Verapamil and Nifedipine

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Summary. The effect of the slow channel inhibitors verapamil ($2.2 \times 10^{-7}$ mol/l and $2.2 \times 10^{-6}$ mol/l) and nifedipine ($5 \times 10^{-8}$ mol/l) on atrioventricular action potential and automaticity was studied in small specimens prepared from the av-node of rabbits.

1. Nodal and NH-action potentials proved susceptible to isoprenaline and responded to $2.3 \times 10^{-9}$ mol/l with a $V_{\text{max}}$ increase of $44.0 \pm 6.4\%$ and $40.0 \pm 8.5\%$, respectively. $V_{\text{max}}$ and overshoot were linearly related to the logarithm of external Ca and Na concentration. The overshoot of N-cells changed by 18 mV and the overshoot of NH-cells by 16 mV per tenfold variation in the external Ca concentration. A similar strong deviation from the theoretical value derived from the Nernst equation appeared on varying the external Na concentration, yielding a slope factor of overshoot of 23 mV in N-cells and of 32 mV in NH-cells per tenfold concentration change.

2. Verapamil at the lower concentration abolished pacemaker activity of N-cells within $21.1 \pm 4.8$ min and decreased $V_{\text{max}}$ to $38.9 \pm 5.8\%$ of the initial control value, whilst overshoot declined by $13.3 \pm 2.0$ mV. The same response was obtained in NH-cells. Higher concentrations led to cessation of automaticity within $7.3 \pm 1.3$ min but the $V_{\text{max}}$ reduction observed just before pacemaker arrest did not differ from that produced by the lower verapamil concentration. Washout experiments of as long as 60 min did not remove the inhibitory drug action regardless of the concentration applied.

3. Stimulation experiments performed after the occurrence of pacemaker arrest indicated that verapamil induces both a resting-state and a use-dependent block. In the absence of major changes of threshold potential, the latter developed exponentially. The interstimulus interval strongly affected the use-dependent block in that a decrease both enhanced the strength and diminished the time constant of development, and vice versa. Removal of the use-dependent block needed 20—30 s. The strength of both types of block increased with increasing exposure to verapamil until a complete resting-state block abolished excitability of the atrioventricular cell.

4. Elevated Ca concentrations as tested up to 5 mmol/l restored pacemaker activity and increased $V_{\text{max}}$ but did not abolish the use-dependent block. In a train of spontaneously generated action potentials, $V_{\text{max}}$ declined from beat to beat until complete blockade occurred which initiated a resting period of several seconds.

5. Nifedipine caused a pacemaker arrest in N-cells within $12.5 \pm 3.0$ min and a reduction of $V_{\text{max}}$ to $54.8 \pm 14.0\%$. The same response was obtained in NH-cells. Like verapamil, nifedipine is capable of blocking $V_{\text{max}}$ already under resting conditions but the inhibitory drug action becomes strongly enhanced by repetitive stimulation. Excess Ca (5 mmol/l) weakened the rate-independent block but did not prevent the appearance of the use-dependent block.

Key words: AV-Node - Slow inward current - Na - Ca - Pacemaker action potential - Verapamil - Nifedipine

Introduction

The electrophysiological effects of verapamil and nifedipine in the heart have been well established by a large number of studies during the last few years. As first demonstrated by Kohlhardt et al. (1972) and Kohlhardt and Fleckenstein (1977), both compounds, although being completely different in chemical structure, strongly inhibit the cardiac slow inward current ($I_{\text{ss}}$). This, however, does not necessarily imply that the underlying mode of action is identical. In fact, verapamil for instance delays the time course of recovery from inactivation (Kohlhardt and Mnich 1978) by producing a significant shift of the curve relating recovery to membrane potential to higher values. Nifedipine fails to change the recovery kinetics (Kohlhardt and Fleckenstein 1977). Though verapamil has not been found to selectively affect the $I_{\text{ss}}$ system, as it also influences K currents (Kass and Tsien 1975) either by a direct effect on outward conductance or indirectly by some decrease of intracellular Ca concentration, slow channel inhibitors have proven valuable tools in identifying the nature of ionic currents in excitable membranes. These drugs depress excitability of atrial and ventricular myocardium if the $I_{\text{ss}}$ system mediates the generation of action potentials. Consequently, they gain an antiarhythmic potency when slow response action potentials are basically involved in the development or maintenance of cardiac arrhythmias.

Apart from the sinus node (for review see Irisawa 1978), the av-node seems to represent another cell population in which $I_{\text{ss}}$ mediates the excitation process under physiological conditions. Atrioventricular action potentials are characterized by a very low rate of rise (Hoffman et al. 1958) being correlated with the extremely small conduction velocity within the av-node. They have proven insensitive towards the inhibitor of the fast Na system, TTX (Zipes and Mendez 1973) but respond to Mn ions with a decline in amplitude (Zipes and Mendez 1973). As demonstrated by Wit and
Cranefield (1974), a similar inhibitory effect appears after exposing the av-node to verapamil. The well-known difficulties in maintaining a continuous microelectrode impalement in the commonly used av-preparations are the reason why a more detailed electrophysiological analysis of the influence of verapamil and other slow channel inhibitors is still lacking.

The present study tried to further elucidate the mode of action of the slow channel inhibitors verapamil and nifedipine in nodal (N) and NH-cells. The methodological problem mentioned above has been overcome by utilizing very small specimens of the av-node. Thus, a continuous action potential analysis in one cell over a long period of time was possible.

Another aim of these experiments was to re-examine the significance of $I_N$ for action potential generation since recent results of Ruiz-Ceretti et al. (1978) have revealed the existence of a TTX-sensitive upstream component. The latter is inconsistent with the prevailing assumption that excitation in av-node is predominantly mediated by the $I_N$ system.

### 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 mmol/l), 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 was stepwise reduced in size until the av-node area including a small residue of atrial and ventricular septum had been isolated. Thereafter, the atrial tissue containing the av-node was separated from the interventricular septum. The overwhelming majority of av-node preparations tolerated this isolation in that they continued to beat. After an equilibration period of 30 min, the av-node area was dissected in small specimens of about 0.8 x 0.8 x 0.2 mm in size by means of precision scissors. After an initial period of quiescence lasting 1—3 min, about 40% of the specimens regained their automaticity and maintained a stable rhythm during the subsequent equilibration period of 30 min. Beating preparations were transferred into a lucite chamber in which the electrophysiological experiments were performed. A microsuction device installed at the bottom allowed the fixation of the preparation without any mechanical irritation and strongly favoured stable intracellular microelectrode impalements by minimizing isotonic mechanical activity of the specimens.

Action potentials were measured using flexibly mounted 3 mol/l KC1-filled conventional glass microelectrodes of a resistance between 10 MΩ and 30 MΩ and differentiated by an analogue differentiator yielding the maximal rate of rise ($V_{max}$). Both signals were displayed on a Tektronix storage oscilloscope 564 B and photographed from the screen by a reflex camera.

KCl (3 mol/l) filled glass micropipettes of low electrical resistance (< 1 kΩ) were used as external stimulation electrodes. They were attached to a micromanipulator and placed a small distance (about 0.3 mm) away from the impaled cell on the surface of the preparation. Rectangular pulses of 2 ms in duration and a strength twice threshold, i.e. 6 V at a minimum were applied. Since such a relatively strong electrical stimulus might release vegetative transmitters from their cellular stores, atropine (0.7 x 10⁻⁵ mol/l) and pindolol (1 x 10⁻⁶ mol/l) were added to the solutions when stimulation experiments were performed in order to avoid acetylcholine or catecholamine effects on the pacemaker cell.

The lucite chamber (volume: 1.5 ml) in which the preparations had been brought for electrophysiological analysis was continuously perfused (8.5 ml/min) with oxygenated Tyrode solution (Ca concentration: 1 mmol/l). The specimens proved highly sensitive to pressure or stretch. Thus, pronounced changes of automatic impulse generation sometimes occurred even during microelectrode impalement. Moreover, amplitude and $V_{max}$ of the action potential needed up to 5 min to attain steady-state values after the cell had been punctured. This, together with the observation that $V_{max}$ can vary from one cell to another by about 20%, demands rejection of experiments in which stable microelectrode impalement could not be maintained in a single cell.

All values are given as mean ± MSE.

### Solution

Tyrode solution (composition in mmol/l): NaCl 137; KC1 7.45; CaCl₂ 1.19; NaHCO₃ 11.9; NaH₂PO₄ 11. Bubbled with carbogen (97% O₂; 3% CO₂). pH 7.4. Temperature 34°C.

The following compounds were used: 1. Racemic verapamil (Knoll AG, Ludwigshafen/Rhein, FRG) = α-isopropyl-x-[(N-methyl-N-homoveratril)-γ-aminopropyl]-3,4-dimethoxyphenylacetoniitrile. 2. Nifedipine (Bayer AG, Leverkusen, FRG) = (4-2-nitrophenyl)-2,6-dimethyl-3,5-dicarboxmethoxy-1,4-dihydropyridine 3. Atropine sulfate (Cascan GmbH, Wiesbaden, FRG). 4. Pindolol (Sandor AG, Nürnberg, FRG) = 4-[2-hydroxy-3-(isopropylamino)-propoxy]indole. 5. Isoprenaline (Boehringer Ingelheim, Ingelheim/Rhein, FRG).

### Results

#### 1. Properties of Atrioventricular Action Potentials in Small Specimens of the Av-Node

Subsequent to an equilibration period of about 20—30 min, necessary for recovery from the microdissection trauma, the preparations attained a steady state for at least 180 min. They spontaneously discharged action potentials with properties strongly resembling those recorded from the undissected av-node. According to the results of Hoffman et al. (1959), Paes de Carvalho et al. (1969), Shigeto and Irisawa (1974), Zipes and Mendez (1973), Wit and Cranefield (1974), different types of pacemaker action potentials could be distinguished depending on the origin of the specimens. Nodal (N) cells located in the middle part of the av-node generated from an apparent threshold potential of about —50 mV action potentials with a small upstroke velocity of 10.2 ± 0.54 V/s and an overshoot of 27.0 ± 0.77 mV (n = 40). Another type of action potential can be found in NH-cells. These cells are located in the transitional zone between the lower part of the av-node and the bundle of His. $V_{max}$ of the NH-action potential was significantly larger and amounted to 43.1 ± 1.9 V/s (n = 8) whilst the apparent take off potential ranged between —50 mV and —60 mV. In both N- and NH-cells the upstroke phase of the action potential was exclusively S-shaped in configuration and, in striking contrast to recent studies of Ruiz-Ceretti et al. (1978), lacked a second component (see Fig. 1).

Rate of rise and overshoot of the atrioventricular action potential proved susceptible to changes of extracellular Ca concentration. As tested in the range from 0.4 to 4 mmol/l, both parameters showed a linear dependence on the logarithm of external Ca concentration. A similar strong deviation of $V_{max}$ from the theoretical value of 30.6 mV (at 35°C) as derived from the Nernst equation has already been found by Ruiz-Ceretti et al. (1978). It clearly indicates that Ca ions are not the sole charge carriers of inward current flow during depolarization. This is consistent with the results of Na withdrawal experiments. A decrease of extracellular Na concentration to 37.5 mmol/l (osmotic replacement by sucrose) led to a decline of both $V_{max}$ and overshoot. A linear relationship of $V_{max}$ and overshoot, respectively, on the logarithm of external Na concentration was obtained. But unlike a Nernstian Na electrode, the slope factor of overshoot per decade was 23 mV as compared to 18 mV of $V_{max}$.