Effects of OPC-88117, a new antiarrhythmic agent, on the electrophysiological properties of rabbit isolated hearts

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- 1 Effects of a new antiarrhythmic agent, OPC-88117, on the conduction properties and excitability of Langendorff-perfused rabbit hearts were compared with those of lignocaine.
- 2 OPC-88117 above 10^{-5} M caused a significant prolongation of atrio-His bundle conduction time (A–H interval) as well as His bundle–ventricular conduction time (H–V interval). Lignocaine above 10^{-5} M caused a similar prolongation of H–V interval with a minimum change of A–H interval. The conduction time from His bundle stimulus to the left ventricle (St–LV) at a low frequency (1 Hz) was also prolonged by these drugs around 10^{-4} M.
- 3 OPC-88117 above 3×10^{-6} M prolonged the effective and functional refractory periods of His bundle (ERP_{HB} , FRP_{HB}) as well as the effective refractory period of left ventricular muscle (ERP_{VM}) in a dose-dependent manner. ERP_{HB} , FRP_{HB} and ERP_{VM} were shortened by low concentrations of lignocaine (3×10^{-6} – 10^{-5} M), but were prolonged by high concentrations (3×10^{-5} – 10^{-4} M).
- 4 Lignocaine (3×10^{-6} – 10^{-4} M) induced an upward displacement of the His bundle refractory curve, indicating a greater intraventricular conduction delay for premature excitation. In experiments with OPC-88117, such an upward displacement was observed only at 10^{-4} M.
- 5 These results suggest that the primary electrophysiological effect of OPC-88117 is a lengthening of ventricular refractoriness, and that at high concentrations it may also exert lignocaine-like inhibition of ventricular conduction.

Introduction

OPC-88117 (8-methyl-3-(4-methyl-1-piperazinyl)-2(1H)-quinolinone hydrochloride) is a newly synthesized antiarrhythmic agent. Previous studies on the transmembrane action potential of ventricular muscle have indicated that the primary electrophysiological effect of OPC-88117 is prolongation of action potential duration (APD), and that at high concentrations it also possesses an inhibitory action on the fast sodium channels (Kodama *et al.*, 1988; Toyama *et al.*, 1989). The latter action is similar to lignocaine in terms of its use- and channel state-dependence. Thus, the onset and offset kinetics of the use-dependent block with OPC-88117 are very fast. Furthermore, this substance blocks the sodium channel mainly during the inactivated state. These findings suggest that OPC-88117 is a unique antiarrhythmic drug having characteristics both of Class

III and Class Ib antiarrhythmic drugs (Hauswirth & Singh, 1979; Vaughan Williams, 1984).

In the present study, the effects of OPC-88117 on the cardiac conduction properties and excitability were investigated in the isolated Langendorff-perfused heart of the rabbit, and compared with those of lignocaine, in order to clarify the pharmacological profile of OPC-88117 as an antiarrhythmic drug more extensively.

Methods

Rabbits weighing 1.5 to 2.0 kg were killed by a blow on the head. The heart was quickly removed and a cannula was inserted into the aorta for Langendorff perfusion. The preparation was perfused at a constant flow (20 ml min^{-1}) with Krebs-Ringer solution, gassed with 95% O_2 and 5% CO_2 , of the following composition (mM): NaCl 120.0, KCl 4.0, $CaCl_2$ 1.2,

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Table 1 Effects of OPC-88117 and lignocaine on the atrioventricular conduction

		A-H interval (%)	H-V interval (%)
OPC-88117	10^{-6} M	103.5 ± 1.7	103.1 ± 0.1
	3×10^{-6} M	104.7 ± 0.8	103.1 ± 0.1
	10^{-5} M	$109.2 \pm 0.4^*$	$108.2 \pm 0.7^*$
	3×10^{-5} M	$121.0 \pm 1.5^*$	$118.6 \pm 1.5^*$
	10^{-4} M	A-H block (2/5)	H-V block (3/5)
Lignocaine	10^{-6} M	99.9 ± 3.6	101.4 ± 0.6
	3×10^{-6} M	96.8 ± 1.1	103.7 ± 2.1
	10^{-5} M	100.1 ± 2.7	$111.6 \pm 3.3^*$
	3×10^{-5} M	106.9 ± 2.6	$124.7 \pm 6.7^*$
	10^{-4} M	$120.7 \pm 4.4^*$	$164.3 \pm 9.8^*$
	3×10^{-4} M	—	H-V block (5/5)

Values are presented as percentage of control (means $\pm$ s.e., $n = 5$) for each drug. A-H interval, atrio-His bundle conduction time; H-V interval, His bundle-ventricle conduction time. Data were obtained 15 min after application of the drug at the respective concentration. * Significantly different from control at $P < 0.05$.

MgSO₄ · 7H₂O 1.3, NaH₂PO₄ 1.2, NaHCO₃ 24.2 and glucose 5.5 (pH 7.4). The temperature of the perfusate was maintained at 34°C. Bipolar stainless steel electrodes with an interpolar distance of 1.0 mm were inserted through a small incision made in the right atrium so as to record His bundle electrograms (HBE). Another pair of bipolar recording electrodes was placed on the epicardial surface of left ventricle (LV) near the apex. The signals were amplified at a frequency response from 100 to 500 Hz with a time constant of 0.03 s and displayed on a digital storage oscilloscope (Tektronix 5223). A pair of stimulating electrodes (interpolar distance, 1.0 mm) made of stainless wires were placed on the right atrium close to the sinus node region.

In experiments to test the ventricular conduction properties and refractoriness at a slow basic driving frequency, the lower part of the A-V node was ligated with a fine silk thread to produce A-V block, and the heart was driven through a pair of contiguous bipolar electrodes placed at the proximal end of the His bundle (Osaka *et al.*, 1988). When the refractoriness of ventricular working myocardium was tested, the heart was stimulated through bipolar electrodes on the epicardial surface of the left ventricle.

Parameters measured were as follows: atrio-His bundle conduction time (A-H interval), His bundle-ventricular conduction time (H-V interval), conduction time from His bundle stimulus to the left ventricle (St-LV interval), effective refractory period of His bundle (ERP_{HB}), functional refractory period of His bundle (FRP_{HB}) and effective refractory period of left ventricular muscle (ERP_{VM}).

A-H and H-V intervals were measured under constant right atrial stimulation at a cycle length of 400 ms. St-LV interval was measured under constant His-bundle stimulation at a cycle length of 1000 ms.

The pulses for stimulation were 2 ms in duration and twice the diastolic threshold in intensity.

To obtain ERP_{HB} and FRP_{HB} a premature stimulus (S₂) was applied through His bundle stimulating electrodes with various coupling intervals, at every tenth basic stimulus (S₁) and a cycle length of 1000 ms, by means of a programmable stimulator. The shortest S₁-S₂ interval captured by left ventricular response (LV) was defined as ERP_{HB}. The FRP_{HB} was defined as the shortest interval between two successive LV excitations. A similar extrastimulus method was used to obtain the ERP_{VM}; the tenth basic stimulus (S₁) at a cycle length of 1000 ms to the left ventricle was followed by a premature stimulus (S₂), and the shortest S₁-S₂ interval captured by ventricular responses was defined as ERP_{VM}.

Control measurements were performed after an equilibration period of 60 min. The effects of drugs were measured 15 min after the heart was perfused with a solution containing OPC-88117 (10^{-6} – 10^{-4} M), or lignocaine (10^{-6} – 3×10^{-4} M). Values are expressed as means $\pm$ s.e. unless otherwise stated. Data were analysed by *t*-test, analysis of variance and Dunnett's test. Differences were considered significant at $P < 0.05$.

Results

Atrio-ventricular conduction

Effects of OPC-88117 and lignocaine on A-H and H-V intervals were tested in each of five preparations, under right atrial stimulation at a constant cycle length of 400 ms. The average control values of A-H and H-V intervals in all 10 preparations were 52.2 ± 2.1 ms and 31.8 ± 0.6 ms, respectively.

Figure 1 Effects of OPC-88117 on the effective and functional refractory periods of His bundle (ERP_{HB}, FRP_{HB}). Ordinate scale: percentage change from control. Values presented are means of five preparations for OPC-88117 (●; ERP_{HB}, ▲; FRP_{HB}), and five for lignocaine (○; ERP_{HB}, △; FRP_{HB}). Vertical lines show s.e.mean. Data were obtained 15 min after application of the drug at the respective concentrations. *Significantly different from control at $P < 0.05$.

OPC-88117 at concentrations $\leq 3 \times 10^{-6}$ M caused no significant changes in these parameters. At 10^{-5} M OPC-88117, a significant prolongation of both A-H and H-V intervals was observed

Figure 2 Effects of OPC-88117 (●) and lignocaine (○) on the conduction time from the His bundle stimulus to the left ventricle (St-LV). Ordinate and abscissa scales are as in Figure 1. Values presented are mean of five preparations for each drug with vertical lines showing s.e.mean. Data were obtained 15 min after application of the drug at the respective concentrations. *Significantly different from control at $P < 0.05$.

Figure 3 Effects of OPC-88117 on the refractory curve of His bundle. Ordinate scale: the interval between two captured ventricular responses with basic and premature His bundle stimuli (LV₁ - LV₂). Abscissa scale: the coupling interval between the basic and premature stimuli (S₁ - S₂). Data were obtained before (○), and 15 min after application of the drug at 3×10^{-6} M (●), 10^{-5} M (△), 3×10^{-5} M (▲) and 10^{-4} M (□). ERP_{HB}: effective refractory period of His bundle; FRP_{HB}: functional refractory period of His bundle.

(Table 1). The prolongation was further enhanced at 3×10^{-5} M. At 10^{-4} M OPC-88117, H-V block was induced in three out of five hearts, and the remaining two showed A-H block. Lignocaine at 1 to 3×10^{-5} M caused a significant and dose-dependent prolongation of H-V interval without affecting the A-H interval (Table 1). At 10^{-4} M lignocaine, a slight but significant prolongation of A-H interval was also observed. The higher concentration (3×10^{-4} M) of lignocaine always induced H-V block.

Conduction properties and refractoriness of the ventricle

Effects of the two drugs on ventricular conduction properties and refractoriness were examined in the hearts where A-V block was artificially produced by ligation. Under His bundle stimulation at a basic cycle length (S₁-S₁) of 1000 ms, the control value of St-LV interval was 34.5 ± 1.7 ms ($n = 10$), and those of ERP_{HB} and FRP_{HB} were 327.8 ± 5.9 ms ($n = 10$) and 356.0 ± 4.7 ms ($n = 10$), respectively.

OPC-88117 at concentrations ranging from 3×10^{-6} M to 3×10^{-5} M caused a dose-dependent prolongation of ERP_{HB} and FRP_{HB} without affecting St-LV intervals for basic stimuli (Figures 1 and 2). At 3×10^{-5} M, ERP_{HB} and FRP_{HB} were prolonged by $33.1 \pm 4.5\%$ ($n = 5$) and $27.6 \pm 3.5\%$ ($n = 5$)

Figure 4 Effects of lignocaine on the refractory curve of His bundle. Ordinate and abscissa scales are as in Figure 3. Data were obtained before (○), and 15 min after application of the drug at 10^{-5} M (●), 3×10^{-5} M (△) and 10^{-4} M (▲).

respectively. At 10^{-4} M OPC-88117, a slight prolongation of St-LV interval was observed in addition to a further prolongation of ERP_{HB} and FRP_{HB} .

Figures 3 and 4 illustrate refractory curves of His bundle. When the coupling interval of premature stimulus ($S_1 - S_2$) was shortened in steps in the control preparations, the intervals between the captured left ventricular responses ($LV_1 - LV_2$) were shortened by similar extents down to $S_1 - S_2$ intervals of 370–380 ms as shown by the data plotted on the identical line. Further shortening of $S_1 - S_2$ resulted in a sharp upward deflection of the curve due to a conduction delay of S_2 excitation. The minimum value of $S_1 - S_2$ and that of $LV_1 - LV_2$ on the curve indicate ERP_{HB} and FRP_{HB} respectively.

In preparations treated with OPC-88117 at concentrations ranging from 3×10^{-6} M to 3×10^{-5} M, the refractory curve shifted towards the right upper direction, giving rise to a dose-dependent prolongation of ERP_{HB} and FRP_{HB} . There was no displacement of the refractory curve from the identical line for the data with $S_1 - S_2$ longer than that causing FRP_{HB} . At 10^{-4} M OPC-88117, however, an appreciable upward displacement of the refractory curve from the identical line was observed.

Lignocaine caused more complex changes of the refractory curve (Figure 4). Thus, at concentrations of $1-3 \times 10^{-5}$ M, a marked shortening of ERP_{HB} and FRP_{HB} was accompanied by an upward displacement of the refractory curve from the identical line. At 10^{-4} M lignocaine, both ERP_{HB} and FRP_{HB} were prolonged to the values above control with a further upward displacement of the refractory curve.

Figure 5 Effects of OPC-88117 (●) and lignocaine (○) on the effective refractory period of left ventricular muscle (ERP_{VM}). Ordinate and abscissa scales are as in Figure 1. Values presented are means of five preparations for each drug with vertical lines showing s.e.mean. Data were obtained 15 min after application of the drug at the respective concentrations. * Significantly different from control at $P < 0.05$.

Figure 5 shows the effects of OPC-88117 and lignocaine on ERP_{VM} . The control value of ERP_{VM} was 322.5 ± 4.7 ms ($n = 10$). OPC-88117 above 3×10^{-6} M caused a significant prolongation of ERP_{VM} in a concentration-dependent manner. At 10^{-4} M OPC-88117, ERP_{VM} was prolonged by $60.3 \pm 6.8\%$ ($n = 10$). Lignocaine caused biphasic changes of ERP_{VM} as in the case of ERP_{HB} and FRP_{HB} (Figure 1). Thus, ERP_{VM} was shortened by a low concentration of lignocaine (3×10^{-6} M) while it was prolonged by high concentrations (3×10^{-5} M, 10^{-4} M).

Discussion

The present study showed that OPC-88117 above 10^{-5} M prolonged H-V as well as A-H intervals. At 10^{-4} M OPC-88117, St-LV interval was also prolonged. Such negative dromotropic effects of OPC-88117 on the ventricular conduction may be attributed to the inhibitory action of this substance on the cardiac fast sodium channels.

In our previous experiments in guinea-pig ventricular muscle constantly stimulated at 1.0 Hz, OPC-88117 at concentrations above 3×10^{-5} M caused a significant decrease in the maximum upstroke velocity ($\dot{V}_{max}$) of action potential without affecting the resting potential level. This inhibition of $\dot{V}_{max}$ by OPC-88117 was shown to be augmented at higher stimulation frequencies (Kodama *et al.*, 1988;

Toyama *et al.*, 1989). The different threshold concentration of OPC-88117 required to prolong H-V and St-LV intervals in the rabbit hearts can be explained by the frequency-dependent inhibition of $\dot{V}_{max}$. The H-V interval was measured under right atrial stimulation at a cycle length of 400 ms (2.5 Hz), whereas the St-LV interval was measured under His bundle stimulation at a cycle length of 1000 ms (1 Hz). At the higher stimulation frequency, OPC-88117 even at relatively low concentrations may have caused a considerable decrease in $\dot{V}_{max}$ of action potential, sufficient to reduce the conduction velocity in Purkinje and ventricular muscle fibres.

The lignocaine-induced prolongation of H-V and St-LV may also be interpreted by similar mechanisms. It has been shown by previous investigators that lignocaine at concentrations above 10^{-5} M decreases $\dot{V}_{max}$ of action potential in Purkinje (Davis & Temte, 1969; Bean *et al.*, 1982) as well as in ventricular muscle fibres (Chen *et al.*, 1975; Grant *et al.*, 1980) especially when the tissue was stimulated at rates higher than 1.0 Hz. The frequency-dependent inhibition of $\dot{V}_{max}$ is known to reflect the 'use-dependent' sodium channel blocking action of this drug (Grant *et al.*, 1984; Hondeghem & Katzung, 1984).

The potency of OPC-88117 for the inhibition of ventricular conduction is comparable to lignocaine on a molar basis. As regards the inhibition of A-V nodal conduction, however, OPC-88117 is more potent than lignocaine. Since the electrical activity of A-V node depends primarily on the slow calcium inward current (I_{Si}) (Carmeliet & Vereecke, 1979), OPC-88117, unlike lignocaine, might inhibit I_{Si} as well. Further experimental studies are required to prove this possibility.

The present study has revealed that OPC-88117 causes a marked dose-dependent prolongation of refractory periods of His bundle and left ventricular muscle. The threshold concentration for this action (3×10^{-6} M) was lower than that for the negative dromotropic effect. In guinea-pig papillary muscles, OPC-88117 above 3×10^{-6} M was shown to prolong the action potential duration (APD) in a dose-dependent manner (Kodama *et al.*, 1988; Toyama *et al.*, 1989). The prolongation of ERP_{HB} , FRP_{HB} and ERP_{VM} is, therefore, most likely due to the prolongation of APD in Purkinje and ventricular muscle fibres.

The refractory curves of His bundle in hearts were not displaced by treatment with OPC-88117 at concentrations ranging from 3×10^{-6} M to 3×10^{-5} M (Figure 3), indicating no substantial conduction delay of premature excitation compared with the basic excitation. Accordingly, a prolongation of the 'time-dependent refractoriness', due to the delayed recovery of excitability after full repolarization of

action potential, is unlikely with OPC-88117 at concentrations up to 3×10^{-5} M.

Effects of lignocaine on the refractory periods of His bundle and left ventricular muscle are different from those of OPC-88117. ERP_{HB} , FRP_{HB} and ERP_{VM} were shortened by lignocaine at concentrations around 10^{-5} M, whereas they were prolonged at 10^{-4} M. An upward displacement of the refractory curve from the control line was observed at all the concentrations tested, but the extent of displacement was greater at the higher dose. These complex changes may be explained by a combination of APD shortening and a lengthening of the time-dependent refractoriness. It has been demonstrated in Purkinje fibres (Weld & Bigger, 1975) and ventricular muscle preparations (Chen *et al.*, 1975; Grant *et al.*, 1980) that lignocaine causes a marked delay in the recovery kinetics of $\dot{V}_{max}$ during the diastolic interval after full repolarization of action potential. This slow recovery of $\dot{V}_{max}$ at the resting potential level has been attributed to the dissociation of the lignocaine molecule from the sodium channel receptors, leading to a slow recovery of sodium channel availability (Grant *et al.*, 1984; Hondeghem & Katzung, 1984). In the presence of lignocaine, therefore, premature stimuli would be expected to induce action potentials with very low $\dot{V}_{max}$, which would be propagated very slowly to the surrounding myocardium, or would be insufficient to be propagated. At high concentrations of lignocaine, a prolongation of the time-dependent refractoriness due to such a mechanism is thought to overcome the shortening of APD, giving rise to a net prolongation of refractory periods.

In preparations treated with OPC-88117 10^{-4} M, an upward displacement of the refractory curve was observed, as in the case of lignocaine. This is consistent with our previous experiments in guinea-pig papillary muscles, where a marked lignocaine-like delay of the recovery of $\dot{V}_{max}$ after repolarization was demonstrated in preparations treated with OPC-88117 10^{-4} M (Toyama *et al.*, 1989).

The effective plasma concentrations of OPC-88117 needed to suppress ventricular arrhythmias in dogs are 0.5 – $1.0 \mu\text{g ml}^{-1}$, corresponding to 1.7 – 3.3×10^{-6} M (unpublished data). Accordingly, it seems reasonable to conclude that the primary electrophysiological effects of OPC-88117 may be a lengthening of ventricular refractoriness through APD prolongation. At very high concentrations, this drug may also exert an inhibition of ventricular conduction through a lignocaine-like sodium channel blocking action.

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References

- BEAN, B.P., COHEN, C.J. & TSIEN, R.W. (1982). Block of cardiac sodium channels by TTX and lidocaine: Sodium current and $V_{\max}$ experiments. In *Normal and Abnormal Conduction in the Heart*. ed. Paes de Carvalho, A., Hoffman, B.F. & Lieberman, M. pp. 198–209. Mount Kisco, N.Y.: Futura.
- CARMELIET, E. & VEREECKE, J. (1979). Electrogenesis of the action potential and automaticity. In *Handbook of Physiology, The Cardiovascular System*, Vol. 1. ed. Berne, R.M., Sperelakis, N. & Geiger, S.R. pp. 269–334. Bethesda, Maryland: American Physiology Society.
- CHEN, C., GETTES, L. & KATZUNG, B. (1975). Effect of lidocaine and quinidine on steady-state characteristics and recovery kinetics of $(dV/dt)_{\max}$ in guinea pig ventricular myocardium. *Circ. Res.*, **37**, 20–29.
- DAVIS, L.D. & TEMTE, J.V. (1969). Electrophysiological actions of lidocaine on ventricular muscle and Purkinje fibers. *Circ. Res.*, **24**, 639–655.
- GRANT, A.O., STRAUSS, L.J., WALLACE, A.G. & STRAUSS, H.C. (1980). The influence of pH on the electrophysiological effects of lidocaine in guinea pig ventricular myocardium. *Circ. Res.*, **47**, 542–550.
- GRANT, A.O., STARMER, C.F. & STRAUSS, H.C. (1984). Antiarrhythmic drug action. Blockade of the inward sodium current. *Circ. Res.*, **55**, 427–439.
- HAUSWIRTH, O. & SINGH, B.N. (1979). Ionic mechanism in heart muscle in relation to the genesis and the pharmacologic control of cardiac arrhythmias. *Pharmacol. Rev.*, **30**, 5–63.
- HONDEGHEM, L.M. & KATZUNG, B.G. (1984). Antiarrhythmic agents: The modulated receptor mechanism of action of sodium and calcium channel-blocking drugs. *Ann. Rev. Pharmacol. Toxicol.*, **24**, 387–423.
- KODAMA, I., MORIMOTO, M. & TOYAMA, J. (1988). Electrophysiological effects of OPC-88117, a new antiarrhythmic agent, on isolated guinea pig ventricular muscle. *Environmental Medicine*, **32**, 131–138.
- OSAKA, T., KODAMA, I., TOYAMA, J. & YAMADA, K. (1988). Effects of bepridil on ventricular depolarization and repolarization of rabbit isolated hearts with particular reference to its possible proarrhythmic properties. *Br. J. Pharmacol.*, **93**, 775–780.
- TOYAMA, J., KODAMA, I., HONJO, H. & KAMIYA, K. (1989). Electrophysiological effects of OPC-88117, a new antiarrhythmic agent on papillary muscles and single ventricular myocytes isolated from guinea pig hearts. *Br. J. Pharmacol.*, **98**, 177–185.
- VAUGHAN WILLIAMS, E.M. (1984). A classification of antiarrhythmic actions reassessed after a decade of new drugs. *J. Clin. Pharmacol.*, **24**, 127–147.
- WELD, F.M. & BIGGER, J.T. JR. (1975). Effects of lidocaine on the early inward transient current in sheep cardiac Purkinje fibers. *Circ. Res.*, **37**, 630–639.

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