Negative chronotropic effect of a novel class III antiarrhythmic drug, UK-68,798, devoid of β -blocking action on isolated guinea-pig atria

Tao Yang, Pål M. Tande & ¹Helge Refsum

Department of Medical Physiology, Institute of Medical Biology, University of Tromsø, N-9000 Tromsø, Norway

1 The chronotropic effects of a novel class III antiarrhythmic drug, UK-68,798, and the β -adrenoceptor blocker, propranolol, for comparison, were studied on spontaneously beating right atria isolated from guinea-pigs in the absence and presence of increasing concentrations of isoprenaline (10^{-10} – 10^{-4} M).

2 UK-68,798 (10^{-9} – 10^{-5} M) decreased spontaneous atrial rate by 6–21%. Propranolol (10^{-8} – 10^{-6} M) also had a negative but significantly smaller chronotropic effect.

3 UK-68,798 dose-dependently reduced the maximal positive chronotropic effect induced by isoprenaline, but without significantly shifting the concentration-response curve for isoprenaline in a parallel fashion. A pD_2 value of 5.88 was obtained. As expected, propranolol displayed a competitive inhibition with a pA_2 value of 8.21.

4 The results demonstrate a negative chronotropic effect of UK-68,798, which is not associated with a β -adrenoceptor blocking action. We suggest that the negative chronotropic effect is linked with potassium channel blockade and thereby the class III antiarrhythmic action of UK-68,798.

Keywords: Class III antiarrhythmic; automaticity; sinus node; non-competitive antagonism; I_K

Introduction

UK-68,798, i.e., N-[4-(2-{2-[4-(methanesulphonamide)phenoxy]-N-methylethylamino}ethyl)phenyl] methanesulphonamide, is a newly synthesized class III antiarrhythmic compound. It potently prolongs action potential duration and effective refractory period in isolated cardiac muscle and the intact heart, probably by selective inhibition of the delayed rectifying potassium current, I_K (Gwilt *et al.*, 1989a; Tande *et al.*, 1990b).

A negative chronotropic effect of UK-68,798 has also been noted both *in vitro* (Tande *et al.*, 1990b) and *in vivo* (Gwilt *et al.*, 1989b; Yang *et al.*, 1990). Other drugs with class III antiarrhythmic action like amiodarone (Kadoya *et al.*, 1985) and sotalol (Campbell, 1987) also inhibit sinus node function which may give bradycardia. It has been debated whether the negative chronotropic effect of amiodarone and sotalol could be due to their effect on repolarization alone, i.e. linked with the class III action, or other actions like antiadrenoceptor effects (Polster & Broekhuysen, 1975; Tande & Refsum, 1990a).

The present study was therefore designed to assess further the chronotropic effect of UK-68,798 and to investigate whether it has any β -adrenoceptor blocking action. Experiments were performed on guinea-pig right atria. Propranolol, a typical β -adrenoceptor blocker, was used for comparison.

Methods

Right atria with sinoatrial node tissue were quickly dissected from the hearts of female guinea-pigs ($n = 19$, weighing 200–300 g) and placed in a water-jacketed organ bath containing 20 ml Tyrode solution gassed with 95% O_2 plus 5% CO_2 kept at $32 \pm 0.5^\circ C$. The solution had the following composition (mm): Na^+ 141.2, K^+ 5.3, Cl^- 129.4, Ca^{2+} 2.0, HCO_3^- 20, $H_2PO_4^-$ 1.2, Mg^{2+} 1.0, glucose 10. The pH was adjusted to 7.4. The atrial preparations were allowed to beat spontaneously throughout the experiments. To record spontaneous atrial rate (SAR), isometric contractions were measured with a force displacement transducer (Type FT03C, Grass Instru-

ments Co, Quincy, Mass) connected to a dynograph recorder (Type 511A, Beckman Instruments Inc, Schiller Park, Ill). A preload of 500 mg was put on the preparations.

Experimental protocol

In order to study any possible β -blocking activity of UK-68,798, concentration-response (CR) curves for isoprenaline were obtained in 14 spontaneously beating right atria. Cumulative doses of isoprenaline (10^{-10} to 10^{-4} M) were introduced after an equilibration period of 60 min, each increment being added when the preceding one had produced its maximum effect. In this way, a control CR curve was obtained. Then, the preparation was repeatedly washed with the Tyrode solution for 1 h (five to six times), and the spontaneous frequency stabilized. In 7 experiments, 10^{-8} and 10^{-7} M UK-68,798 were added to the organ bath 20 min before repeating the CR curves for isoprenaline. In 5 of these experiments 10^{-9} M UK-68,798 was given 20 min before 10^{-8} M but without giving isoprenaline. In the other 7 experiments UK-68,798 at 10^{-6} and 10^{-5} M was given. For reference, the same procedure was repeated with propranolol (10^{-8} , 10^{-7} and 10^{-6} M) in 5 experiments. The direct effects on SAR of increasing concentrations of UK-68,798 (10^{-9} – 10^{-5} M) or propranolol (10^{-8} – 10^{-6} M) could thereby also be obtained before establishing the CR curves for isoprenaline.

Drug preparation

UK-68,798 was a gift from Pfizer Central Research (Sandwich, Kent, U.K.). ($\pm$)-Propranolol hydrochloride was obtained from Aldrich-Chemie (Steinheim, F.R.G.). Isoprenaline sulphate was obtained from NAF-Laboratory (Oslo, Norway). Stock solutions (10^{-3} M) of UK-68,798 (acidified to pH 3) and propranolol in distilled water were kept at $-18^\circ C$. Dilutions were made in Tyrode solution during the course of each experiment.

Calculations and statistics

The effects on SAR of increasing concentrations of UK-68,798 or propranolol were calculated as percentages of change from baseline value obtained prior to addition of each concentration.

¹ Author for correspondence.

The concentration of isoprenaline eliciting half maximal response (EC_{50}) was obtained from graphical plots of the CR curve from each experiment. The EC_{50} value was expressed as pD_2 value ($pD_2 = -\log EC_{50}$, negative logarithm of molar concentration of agonist that caused half maximal response) (Van den Brink, 1977). For the competitive antagonism, pA_2 values were calculated from the following equation (Mackay, 1978):

$$pA_2 = \log(DR - 1) - \log[B] \quad (1)$$

where DR represents the agonist dose-ratio and $[B]$ represents the molar concentration of the antagonist. The pA_2 value is defined as the negative logarithm of molar concentration of antagonist that caused a twofold shift of the CR curve for agonist. For the non-competitive antagonism, pD'_2 values were determined according to the following equation (Van den Brink, 1977):

$$pD'_2 = -\log[B] + \log(E_A/E_{AB} - 1) \quad (2)$$

where $[B]$ represents the molar concentration of antagonist, E_A represents the effect of agonist in the absence of antagonist and E_{AB} represents the effect of agonist in the presence of antagonist. The pD'_2 value is equal to the negative logarithm of molar concentration of antagonist that caused half reduction of the CR curve for agonist.

Mean values $\pm$ s.e.mean are used to express the data. Two-tailed Student's t tests for either paired or unpaired observations were used to compare the results. $P < 0.05$ was considered statistically significant.

Results

Negative chronotropic effects of UK-68,798 and propranolol

The baseline SAR of the guinea-pig isolated right atria was 157 ± 5 beats min^{-1} ($n = 19$). Increasing concentrations of UK-68,798 (10^{-9} – 10^{-5} M) decreased SAR by 6% ($n = 5$), 13%, 19% ($n = 7$), 20% and 21% ($n = 7$), respectively (Figure 1). Thus, even at a concentration of 10^{-8} M there was a significant negative chronotropic effect of UK-68,798 and at 10^{-7} M it seemed to have reached its maximum. In another group of 5 experiments, propranolol (10^{-8} – 10^{-6} M) decreased SAR to a certain extent but significantly less than UK-68,798 at the same concentrations (Figure 1).

Figure 1 Effects of UK-68,798 (solid columns, 10^{-9} M, $n = 5$; 10^{-8} – 10^{-7} M, $n = 7$; 10^{-6} – 10^{-5} M, $n = 7$) and propranolol (open columns, 10^{-8} – 10^{-6} M, $n = 5$) on spontaneous atrial rate of guinea-pig isolated rat atria 20 min after addition of each concentration. Mean values (s.e.mean shown by vertical bars) are calculated as percentages from baseline values. * $P < 0.05$ as compared with control values (paired t test based on real values); † $P < 0.05$ as compared with propranolol (unpaired t test based on the percentage changes).

Changes in the concentration-response curves for the isoprenaline-induced positive chronotropic response

Isoprenaline, used as β -adrenoceptor agonist, significantly increased SAR. Before addition of UK-68,798 or propranolol, the control pD_2 values of isoprenaline were 8.13 ± 0.08 ($n = 7$) and 8.18 ± 0.08 ($n = 7$) in the two UK-68,798 groups and 8.00 ± 0.12 ($n = 5$) in the propranolol group, respectively, in good agreement with the values reported by others (8.06–8.76) (O'Donnell & Wanstall, 1979; Polster *et al.*, 1975). After addition of UK-68,798, the pD_2 value obtained after each concentration of UK-68,798 was not significantly different from the control value (Table 1). As shown in Figure 2, increasing concentrations of UK-68,798 did not significantly shift the CR curve for isoprenaline to a higher concentration-range in a parallel manner. However, the maximum response of the CR curve for isoprenaline was reduced dose-dependently in the presence of UK-68,798, which is characteristic for non-competitive antagonism. The pD'_2 value calculated for the various concentrations of UK-68,798 was 5.88 ± 0.59 ($n = 14$).

On the other hand, propranolol shifted the CR curve for isoprenaline to the right with no reduction in the maximum response (Figure 3), demonstrating competitive β -adrenoceptor antagonism. As shown in Table 1, the pD_2 value

Figure 2 Cumulative concentration-response curves for the positive chronotropic effects of isoprenaline (10^{-10} – 10^{-5} M) on spontaneously beating guinea-pig right atria in the absence and presence of increasing concentrations of UK-68,798: (○) control ($n = 14$); (●) 10^{-8} M and (□) 10^{-7} M, ($n = 7$); (■) 10^{-6} M and (△) 10^{-5} M, ($n = 7$). Values shown are mean with s.e.mean indicated by vertical bars.

Figure 3 Cumulative concentration-response curves for the positive chronotropic effects of isoprenaline (10^{-10} – 10^{-4} M) on spontaneously beating guinea-pig right atria in the absence and presence of increasing concentrations of propranolol: (○) control; (●) 10^{-8} M; (□) 10^{-7} M and (■) 10^{-6} M ($n = 5$). Values shown are mean with s.e.mean indicated by vertical bars.

Table 1 The effects of various concentrations of UK-68,798 and propranolol on pD_2 , pA_2 , pD'_2 and maximum chronotropic response ($I_{so_{max}}$) of guinea-pig isolated right atria to isoprenaline (10^{-10} – 10^{-4} M)

Conc. (M)	n	pD_2	pA_2	pD'_2	$I_{so_{max}}$ (%)
UK-68,798					
0	7	8.13 ± 0.08			100
10^{-8}	7	7.82 ± 0.11		7.24 ± 0.30	92 ± 3
10^{-7}	7	7.84 ± 0.15		6.38 ± 0.27	$86 \pm 4^*$
0	7	8.18 ± 0.08			100
10^{-6}	7	7.91 ± 0.08		5.33 ± 0.17	$78 \pm 7^*$
10^{-5}	7	7.89 ± 0.10		4.55 ± 0.15	$71 \pm 6^*$
Propranolol					
0	5	8.00 ± 0.12			100
10^{-8}	5	$7.51 \pm 0.12^*$	8.25 ± 0.17		99 ± 2
10^{-7}	5	$6.82 \pm 0.20^*$	8.31 ± 0.10		98 ± 1
10^{-6}	5	$5.93 \pm 0.12^*$	8.06 ± 0.22		97 ± 2

All values represent the mean $\pm$ s.e.mean of n experiments.

* $P < 0.05$ compared to control (0) value. The explanations for pD_2 , pA_2 and pD'_2 are given in Methods.

for isoprenaline was significantly decreased. A pA_2 value of 8.21 ± 0.08 ($n = 5$) was obtained for propranolol.

Discussion

The spontaneously beating guinea-pig right atrial preparation has previously been shown to be a reliable method for studying chronotropic effects of cardiac drugs (Kline & Buckley, 1972). It has been demonstrated that guinea-pig atria, unlike cat atria, contain a single β -adrenoceptor type (β_1) and not a β -adrenoceptor mixture (β_1 and β_2) (O'Donnell & Wanstall, 1975). Therefore, guinea-pig atria should be a better tissue for assessing the potency of agonists and antagonists of β_1 -adrenoceptors. Thus, in guinea-pig right atria with sinoatrial node tissue, the positive chronotropic effect induced by isoprenaline, a non-selective β -adrenoceptor agonist, is most likely to be mediated through activation of β_1 -adrenoceptors (Spilker *et al.*, 1974; O'Donnell & Wanstall, 1979).

In the present study increasing concentrations of UK-68,798 decreased spontaneous atrial rate. This is in good agreement with our previous results where we, in addition, demonstrated a parallel increase of sinus node recovery time. No effect on the corrected sinus node recovery time indicated that the spontaneous depolarization phase was not affected (Tande *et al.*, 1990b). A negative chronotropic effect of UK-68,798 has also been observed *in vivo* (Gwilt *et al.*, 1989b; Yang *et al.*, 1990). Similar negative chronotropic effects have been described for D- and L-sotalol on guinea-pig (Campbell, 1987) and rabbit (Kato *et al.*, 1986) sinoatrial node action potentials and attributed almost entirely to prolongation of repolarization. D-Sotalol also decreased heart rate in anaesthetized dogs pretreated with propranolol in correlation with the effect on the QT-time (Mortensen *et al.*, 1990).

UK-68,798 selectively prolongs action potential duration and effective refractory period in guinea-pig papillary muscle, an effect considered to result from inhibition of the delayed rectifying potassium current (I_K) (Gwilt *et al.*, 1989a; Tande *et al.*, 1990b). This is also supposed to be the mechanism behind the prolongation of the action potential by sotalol (Carmeliet, 1985). Studies have shown that a similar current, also named I_K , is also one of the ionic currents contributing to the pacemaker potentials of sinoatrial node cells (Noma & Irisawa, 1976; Yanagihara & Irisawa, 1980). Decay of I_K is believed to be a major controlling factor determining the rate of sponta-

neous depolarization (Noma & Irisawa, 1976). Furthermore, subsequent increase in I_K leads to a more rapid repolarization and contributes to the maximum diastolic potential (Brown *et al.*, 1979). Thus, a selective interaction with I_K of UK-68,798 could explain its negative chronotropic effect.

The effect of UK-68,798 on the CR curve for the isoprenaline-induced positive chronotropy indicates a non-competitive inhibition. This implies that UK-68,798 does not interact with the β -adrenoceptor agonist isoprenaline at the same sites on the cellular membrane. The reduction of the maximum response of the CR curve for isoprenaline by UK-68,798 might be explained by the suggestion that inhibition of I_K in pacemaker cells of the sinoatrial node is probably opposite to the mechanism by which isoprenaline induces its positive chronotropic effect. β -Adrenoceptor stimulation may partly accelerate both depolarization and repolarization of the pacemaker cells through increasing I_K (Brown & DiFrancesco, 1980).

Propranolol is a competitive β -adrenoceptor antagonist with membrane stabilizing properties. In isolated cardiac preparations, the membrane stabilizing activity of propranolol has been reported at 10^{-6} M or above (Doggrell & Hughes, 1986). Propranolol has also previously been shown to decrease spontaneous frequency of isolated atria (Nickerson & Collier, 1975). Thus, besides β -adrenoceptor blocking action of propranolol, which may be of significance *in vitro* due to liberation of endogenous catecholamines within atrial tissue (Tande & Refsum, 1988), its membrane stabilizing activity may partly be responsible for the reduced heart rate observed in isolated atrial preparations. In the present study, a parallel rightward shift in the cumulative CR curve for isoprenaline without depression of maximum response occurred in the presence of propranolol. This demonstrates how propranolol significantly antagonizes the accelerated heart rate induced by isoprenaline through competition at the same receptor sites, i.e. competitive β -adrenoceptor antagonism.

In conclusion, UK-68,798 exerts a negative chronotropic effect devoid of β -adrenoceptor blocking action. Class III antiarrhythmic action may thus be linked with negative chronotropy.

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