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COMPARISON OF THE EFFECTS OF A BENZOTRIAZINIUM IODIDE AND QUINIDINE ON GUINEA-PIG HEART

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1 The actions of 2-*n*-propyl-4-*p*-tolylamino-1,2,3-benzotriazinium iodide (TnPBI) and quinidine were compared on guinea-pig heart preparations.

2 Quinidine had negative inotropic and chronotropic effects on guinea-pig isolated atria and on Langendorff hearts: TnPBI had negative inotropic and chronotropic effects on Langendorff hearts, but showed a positive inotropic effect on isolated atria at low dose levels.

3 Both quinidine and TnPBI increased the effective refractory period of guinea-pig atrial preparations.

4 The effects of TnPBI on transmembrane action potentials indicated a mixed class I and class III action according to the classification of Vaughan Williams (1970).

5 These effects are discussed in relation to the antiarrhythmic actions of TnPBI.

Introduction

It has recently been found that a benzotriazinium salt, 2-*n*-propyl-4-*p*-tolylamino-1,2,3-benzotriazinium iodide (TnPBI), has certain pharmacological properties which indicate its potential as an antiarrhythmic agent (French & Scott, 1977; 1978a). This paper compares the effects of TnPBI and quinidine on the rate and force of spontaneously beating quinea-pig atria and Langendorff hearts, the effects on the refractory period of guinea-pig atria, and the changes produced by TnPBI on the intracellularly recorded action potentials of guinea-pig atrial and ventricular cells.

Methods

Effects on spontaneous rate and force of guinea-pig isolated atrial pairs

Each excised atrial pair was mounted in a 30 ml tissue bath containing Locke solution of the following composition (mM): NaCl 154, KCl 5.6, $CaCl_2$ 2.16, glucose 5.5 and $NaHCO_3$ 2.4. The solution was maintained at 32°C and aerated with O_2, and had a pH of 7.2. Contractions of the atria were measured with an Ether UFI strain gauge (57 g model) and displayed on one channel of a Devices M2 recorder. The rate of beating was simultaneously displayed on the second channel via a Devices Instantaneous Rate-meter (Type 2751). Tissues were allowed to settle for 1 h before any drugs were added to the tissue bath. Due to the persistent effects of both TnPBI and quinidine, both drugs were added in a cumulative manner. Each concentration of drug was allowed to act for 30 min before increasing the concentration. Changes in rate and force of contractions were measured after 30 min contact at each concentration.

Effects on ventricular contractions of guinea-pig Langendorff hearts

Guinea-pig isolated hearts were perfused retrogradely through the aorta with oxygenated Locke solution at 32°C. Perfusion was carried out with a Watson-Marlow pump at a rate of 5 ml/min for hearts from guinea-pigs weighing 300 to 400 g. A mercury manometer placed in parallel with the perfusing fluid gave some protection against any pressure increases in the coronary circulation which may occur due to cardiac oedema (Spieckermann, Denkhaus, Gebhard & Sakai, 1977). The force and rate of contractions were measured by the same measuring and recording systems as described in the preceding section. Three concentrations of either TnPBI or quinidine were sequentially perfused through the heart, each for a period of 30 min. After 30 min perfusion with the highest concentration, the perfusate was replaced by drug-free Locke, and the recovery of the tissue from drug effects was monitored over 30 min.

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Effects on the effective refractory period (ERP) of guinea-pig atria

Isolated left atria from guinea-pig hearts were set up in 10 ml organ baths containing Locke Ringer solution at 32°C. The atria were driven with 1 ms pulses at 1 Hz and supramaximal voltage by bipolar platinum electrodes connected, via a stimulus isolation unit (Grass SIU5) to a Grass S48 stimulator. Contractions of the atria were measured with an Ether UFI strain gauge transducer, and after amplification, were displayed on a Gould OS 4000 digital storage oscilloscope. Tissues were allowed to settle for 1 h before any estimates of the refractory period were made. Refractory periods were estimated by applying a second pulse (of the same duration and voltage as the driving pulses) at decreasing intervals after each driving pulse, until the mechanical response to the second pulse was abolished. At this point, the interval between first and second pulses, read off from a Dawe Universal Frequency Meter coupled to the stimulator, was taken as the ERP of the atria. The ERP was then estimated after 10 min contact with drug solutions.

Effects on action potentials in guinea-pig atria and ventricles

A pair of guinea-pig isolated atria was mounted on a Sylgard 182 Resin base in a channel cut in a perspex block, and perfused at 32°C with preoxygenated Locke Ringer solution. Individual cells on the endocardial surface were impaled with glass microelectrodes pulled from 1.2 mm capillary tubing with internal filament and filled by capillary attraction under reduced pressure with 3 M KCl. Electrodes with impedances between 10 to 30 MΩ were used in all experiments. Transmembrane potentials were amplified by a Grass P16 d.c. amplifier and displayed on a Gould OS 4000 digital storage oscilloscope. Stored action potentials were then drawn out on a Beckman pen recorder via a Gould OS 4001 analogue output unit. The atria were stimulated with 3 ms pulses at 10% above their spontaneous frequency by means of bipolar platinum electrodes in contact with the surface of the left atrium.

Strips of ventricular muscle from guinea-pig hearts were mounted in a manner essentially similar to that for atria, and stimulated with 3 ms pulses at 2 Hz and supramaximal voltage.

The atria or ventricular strips were allowed to equilibrate for 30 min to 1 h before any recordings were made. At least six cells were impaled in each tissue before the perfusing Locke solution was replaced by Locke solution containing the drug under test. Each drug solution was perfused for 30 min and then a further six cells were impaled and the action potentials recorded. The parameters measured were the maximum rate of depolarization (MRD); the duration of the action potential at the level of 50% and 90% repolarization (APD_{50}, APD_{90}); the resting membrane potential (RP); the overshoot of the action potential (OS); and the magnitude of the action potential (AP). Conduction velocity was not measured directly, but was estimated as the time interval between the stimulus artefact and the beginning of the action potential. This interval is referred to as the 'conduction time' (CT). All impalements in any experiment were made at points close together and equidistant from the stimulating electrodes: therefore any increase in CT reflects a reduction in conduction velocity.

Table 1 The effects of quinidine and TnPBI on the force and rate of spontaneously beating guinea-pig atrial pairs

	% change in force	*% change in rate*
Quinidine (M)		
1×10^{-5}	-12.3 ± 7.1	-29.0 ± 3.8
2×10^{-5}	-31.9 ± 9.8	-42.5 ± 4.2
4×10^{-5}	-52.6 ± 8.2	-54.1 ± 6.5
TnPBI (M)		
1×10^{-6}	$+20.0 \pm 1.7$	-20.0 ± 2.1
5×10^{-6}	$+9.4 \pm 2.2$	-38.8 ± 2.5
1×10^{-5}	-10.4 ± 4.3	-47.8 ± 2.8

Each value for quinidine is the mean ± s.e. of eight tissues. The values for TnPBI are the means ± s.e. of six tissues. The mean control rate was 190 ± 14 beats/min. No value is given for mean control force of contraction, since this varies widely between individual atrial pairs.

Results

Effects on guinea-pig isolated atrial pairs

The effects of TnPBI and quinidine on the rate and force of spontaneously beating atrial pairs are shown in Table 1. Each figure represents the percentage change in rate or force, measured 30 min after each increase in concentration. TnPBI produced a concentration-dependent reduction in the spontaneous rate of beating, as did quinidine, but TnPBI had a biphasic effect on the force of contractions. At 1×10^{-6} M TnPBI increased the force of contraction by approximately 20% after 30 min. When the concentration was increased to 5×10^{-6} M, the force of contraction decreased from this elevated value, but after 30 min was still above the control value. Only when the concentration was increased to 1×10^{-5} M, did the force of contraction fall below the control value. This biphasic effect was also observed when a single large dose of TnPBI (4×10^{-5} M) was added to the tissue, there being an initial augmentation of the force of contraction (approximately 20 to 25%) which lasted for 5 to 10 min. This augmentation was then followed by a slowly progressing decline in the force of contraction to about 50% of the control value after 30 min. Quinidine showed no such biphasic effect but only a concentration-dependent decline in the force of contractions.

Effects on ventricular contractions in guinea-pig Langendorff hearts

Table 2 shows that TnPBI and quinidine depressed both the force and rate of ventricular contractions in Langendorff heart preparations. This depression was concentration-dependent. TnPBI depressed the force of contractions to a lesser extent than did quinidine but both drugs depressed the rate of beating by approximately the same extent.

Refractory periods of guinea-pig left atria

Table 3 shows the effects of TnPBI and quinidine on the atrial refractory period. Both drugs increased the ERP in a concentration-related manner, but TnPBI was approximately ten times more potent than quinidine on a molar concentration basis.

Effects on transmembrane potentials in guinea-pig atrial and ventricular cells

Table 4 shows the effects of TnPBI and quinidine on action potentials recorded from atrial cells. TnPBI increased both the APD_{50} and APD_{90} values in a concentration-dependent manner. The MRD values were reduced, the effects also being directly related to drug concentration. Quinidine also increased the APD_{50} and APD_{90} values and reduced the MRD, but only the latter effect was concentration-dependent. Table 5 shows that in ventricular cells TnPBI had little effect on APD_{50} values, but did significantly increase the APD_{90} values at all three concentrations used. The effects on ventricular MRD were similar to those on atrial cells, i.e. a concentration-related reduction which was significant at all three concentrations used. Quinidine significantly increased the ventricular APD_{50} and APD_{90} values, although no

Table 2 The effects of quinidine and TnPBI on the ventricular force and rate of guinea-pig Langendorff hearts

	% reduction in force	*% reduction in rate*
Quinidine (M)		
1×10^{-6}	10.1 ± 1.1	14.1 ± 3.2
5×10^{-6}	27.0 ± 2.5	23.2 ± 4.8
1×10^{-5}	41.5 ± 3.1	31.3 ± 3.9
30 min post drug	12.4 ± 4.4	13.3 ± 3.2
TnPBI (M)		
1×10^{-6}	5.4 ± 2.0	6.1 ± 1.7
5×10^{-6}	16.1 ± 3.4	24.8 ± 1.3
1×10^{-5}	25.2 ± 4.2	34.6 ± 0.6
30 min post drug	7.5 ± 2.9	0.9 ± 1.8

All results are the means ± s.e. of 5 preparations. The '30 min post drug' value is the % change from control values 30 min after replacing drug solution with drug-free Locke Ringer. The mean control ventricular rate was 168 ± 11 beats/min for the quinidine experiments and 169 ± 13 beats/min for the TnPBI experiments. No value is given for the mean control force of contraction since this varies widely between individual isolated hearts.

concentration-dependent effect was observable. However, the ventricular MRD was decreased in a concentration-dependent manner, and this decrease was significant at all concentrations used.

TnPBI and quinidine decreased conduction velocity in both atrial and ventricular tissue, as was shown by the increased conduction times (CT) between the stimulus artefact and the beginning of the upstroke of the action potential. TnPBI at concentrations of 1, 5 and 10×10^{-6} M increased the CT by factors of 1.04, 1.19 and 1.32 respectively in atrial tissue, and by factors of 1.04, 1.19 and 1.31 in ventricular tissue. These increases were all significantly different from the control values ($P < 0.05$). For quinidine, 1, 2 and 4×10^{-5} M, the CT was increased by factors of 1.07, 1.41 and 1.32 respectively in atrial tissue. The corresponding increases in ventricular tissue were 1.13, 1.26 and 1.27. Again all increases in CT were significantly different at $P < 0.05$. Neither TnPBI (1×10^{-6} M to 1×10^{-5} M) nor quinidine (1 to 4×10^{-5} M) had any significant effect on the RP, OS or AP values in either atrial or ventricular muscle.

Table 3 The effects of quinidine and TnPBI on the effective refractory period (ERP) of guinea-pig isolated atrial pairs

	% increase in ERP
Quinidine ($\times 10^{-5}$ M)	
1.25	9.4 ± 0.8
2.5	18.8 ± 1.4
5	35.6 ± 3.3
10	53.9 ± 3.1
TnPBI ($\times 10^{-6}$ M)	
1	12.1 ± 0.8
2.5	24.4 ± 2.0
5	48.6 ± 2.6
10	81.3 ± 4.3

All results are the means ±s.e. of 5 tissues. Mean control ERP for quinidine experiments = 175 ± 5 ms. Mean control ERP for TnPBI experiments = 172 ± 4 ms.

Discussion

One unusual property of TnPBI is its ability to increase the force of atrial contractions either at low doses, or on initial application of a dose which eventually leads to a reduction in the contractile force. This is a property which is not shared by quinidine on the same preparation. The positive inotropic effect of TnPBI on atrial tissue is not antagonized by concentrations of propranolol (1 to 5×10^{-6} M) which abolish the effects of adrenaline or isoprenaline. Thus

Table 4 Effects of TnPBI and quinidine on transmembrane potentials in guinea-pig atria

	APD_{50} *Control*	APD_{50} *Treated*	APD_{90} *Control*	APD_{90} *Treated*	*MRD Control*	*MRD Treated*
	(ms)		*(ms)*		*(V/s)*	
TnPBI						
1×10^{-6} M	72 ± 2	83 ± 3	152 ± 6	167 ± 12	137 ± 17	114 ± 14
Ratio	1.14 ± 0.02***		1.10 ± 0.04*		0.83 ± 0.02***	
5×10^{-6} M	70 ± 3	87 ± 3	143 ± 5	167 ± 7	130 ± 15	81 ± 10
Ratio	1.25 ± 0.02***		1.17 ± 0.03***		0.62 ± 0.02**	
1×10^{-5} M	71 ± 7	94 ± 8	133 ± 7	171 ± 10	130 ± 7	62 ± 4
Ratio	1.33 ± 0.06***		1.29 ± 0.05***		0.48 ± 0.02***	
Quinidine						
1×10^{-5} M	67 ± 3	86 ± 4	128 ± 6	156 ± 11	159 ± 12	118 ± 10
Ratio	1.29 ± 0.09**		1.22 ± 0.09*		0.75 ± 0.02	
2×10^{-5} M	80 ± 3	97 ± 3	144 ± 9	172 ± 9	129 ± 13	81 ± 12
Ratio	1.21 ± 0.03***		1.20 ± 0.04***		0.62 ± 0.03***	
4×10^{-5} M	79 ± 3	106 ± 4	142 ± 7	194 ± 9	164 ± 29	77 ± 15
Ratio	1.35 ± 0.03***		1.37 ± 0.02***		0.47 ± 0.05***	

Each result is the mean ±s.e. mean of at least 30 measurements from 5 to 6 tissues. * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$ (Student's paired t test). APD_{50} and APD_{90} are the durations of the action potentials at the level of 50% and 90% repolarization respectively: MRD is the maximal rate of depolarization.

it appears unlikely that this effect is due to the release of endogenous catecholamines from atrial tissue. However, similar effects have been shown for caffeine (De Gubareff & Sleator, 1965), where doses of 2.5 ± 10^{-4} M to 1.5×10^{-3} M increase the force of contraction, while higher doses reduce the force of contraction. These effects were attributed by de Gubareff & Sleator to an interference with calcium mobilization or uptake into the cell, and it may be that TnPBI and caffeine both cause contractions in frog rectus abdominis muscle by releasing intracellular calcium (Muir & Scott, 1977); this supports the suggestion that TnPBI causes changes in calcium ion movements into or within cardiac cells. However, contradictory results were obtained for ventricular contractions which are not augmented by TnPBI. This may reflect some difference between atria and ventricles in the intracellular binding of calcium ions or their transport into the cell.

Both TnPBI and quinidine increased the effective refractory period of isolated atrial pairs. Prolongation of the ERP of cardiac tissue is commonly quoted as being a feature of most antiarrhythmic drugs (Dawes, 1946; Szekeres & Vaughan Williams, 1962). TnPBI is seen to be much more effective than quinidine in this respect, concentrations as low as 1×10^{-6} M causing a 12% increase in ERP, whereas quinidine at a concentration of 1.25×10^{-5} M caused less than 10% increase.

The action of TnPBI on action potentials suggests that the compound has both class I and class III action, i.e. a reduction in MRD, and an increase in action potential duration (Vaughan Williams, 1970). One interesting feature of the results is that quinidine has, in addition to its class I action, an apparent class III effect. Vaughan Williams (1958) suggested that quinidine has little class III action on rabbit atrial APD_{50} at concentrations effective clinically. However, he quotes 10 mg/l has having no significant affect on the APD_{50}. This concentration is equivalent to 2.55×10^{-5} M, which is greater than concentrations which in this study do show a significant prolongation of the action potential duration. Iven & Zetler (1974) have also shown that quinidine consistently prolongs the APD_{50} value at concentrations as low as 7.7×10^{-6} M on guinea-pig atria, while 2.55×10^{-5} M doubled the APD_{50} value. Other actions relevant to antiarrhythmic activity include the prolongation of the ERP, which may be related to the increased APD values, and the decrease in conduction velocity in both atria and ventricles.

These results indicate that TnPBI has properties consistent with those of an antiarrhythmic drug with class I and class III actions: these actions should therefore be of value in the treatment of certain cardiac arrhythmias as has been indicated by the antifibrillatory action of TnPBI on guinea-pig Langendorff hearts (French & Scott, 1978a), and the recent report that TnPBI has antiarrhythmic effects against aconitine-induced arrhythmias in mice and also against adrenaline/halothane-induced arrhythmias in guinea-pigs (French & Scott, 1978b).

Table 5 Effects of TnPBI and quinidine on transmembrane potentials in guinea-pig ventricles

	APD_{50} (ms)		APD_{90} (ms)		MRD (V/s)	
	Control	*Treated*	*Control*	*Treated*	*Control*	*Treated*
TnPBI						
1×10^{-6} M	107 ± 5	108 ± 5	140 ± 3	146 ± 5	177 ± 9	161 ± 9
Ratio	1.01 ± 0.01		1.05 ± 0.01*		0.91 ± 0.02**	
5×10^{-6} M	102 ± 7	107 ± 8	132 ± 6	149 ± 9	187 ± 8	148 ± 8
Ratio	1.05 ± 0.01*		1.13 ± 0.03**		0.75 ± 0.02***	
1×10^{-5} M	110 ± 2	114 ± 3	138 ± 1	154 ± 5	204 ± 12	131 ± 7
Ratio	1.04 ± 0.03		1.12 ± 0.04*		0.64 ± 0.02***	
Quinidine						
1×10^{-5} M	116 ± 5	122 ± 6	161 ± 6	172 ± 8	202 ± 18	167 ± 17
Ratio	1.05 ± 0.02*		1.08 ± 0.02*		0.82 ± 0.04**	
2×10^{-5} M	98 ± 10	113 ± 7	137 ± 12	159 ± 11	190 ± 21	131 ± 15
Ratio	1.12 ± 0.03**		1.17 ± 0.03***		0.71 ± 0.02***	
4×10^{-5} M	132 ± 4	145 ± 5	170 ± 2	188 ± 7	208 ± 15	113 ± 13
Ratio	1.10 ± 0.02***		1.11 ± 0.03*		0.54 ± 0.04***	

Abbreviations, levels of significance and numbers of experiments are all as described in Table 4.

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