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Comparative studies of cardiodepressant drugs on contraction dynamics and electrophysiological parameters of cardiac tissues at pH 7·4 and 9

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The effects of propranolol, amethocaine (tetracaine), lignocaine (lidocaine), procaine and benzocaine on force of contraction and action potential parameters of guinea-pig left atria and papillary muscles have been investigated at pH 7·4 and 9. All the drugs decreased the force of contraction of both preparations. Their biological activities correlated with their lipid/buffer partition coefficients and with their ability to change the surface potential of liposomes. The sequence of activities is as follows: propranolol > amethocaine > lignocaine > benzocaine > procaine. Uncharged benzocaine had no effect on the liposome surface charge. Biological and physicochemical effects obtained at pH 9 were higher than those observed at pH 7·4. Action potential amplitude and V_{max} were decreased by all the drugs except benzocaine. At pH 9, the action potential duration was generally shortened, except by amethocaine and procaine; both caused a prolongation of the action potential at 90% repolarization.

Many drugs are known to depress excitability, force of contraction and action potential propagation in cardiac tissues as well as heart rate. Drugs with such properties are frequently used to treat cardiac arrhythmias and are therefore termed antiarrhythmics. Vaughan Williams (1974) classified drugs with antiarrhythmic properties into four groups among which 'class 1 drugs' comprise quinidine, local anaesthetics and β-adrenoceptor blocking agents with local anaesthetic activity. Those drugs inhibit the function of ion channels (Neher & Steinbach 1978) by changing physicochemical properties of the cell membranes, and consequently depress the excitability of the cardiac tissue. The antiarrhythmic effect is closely related to local anaesthetic properties, and is associated with negative inotropic action (Hellenbrecht et al 1973, 1974; Rauls & Baker 1979), which is an unwanted side effect in their therapeutic use.

The local anaesthetic action may be attributed to three main mechanisms: (i) fluidizing and expanding the phospholipid layer of the cell membrane (Skou 1954; Trudell & Cohen 1975), (ii) incorporation of positive charge in the cell membrane (Hille et al 1975; McLaughlin 1975), and (iii) interaction with membrane-located receptors accessible from the plasmic side (Strichartz 1973; Hille 1977).

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We have demonstrated that local anaesthetics, possessing an amino group, markedly change the surface potential of phospholipid liposomes at pH 4, 7 and 9 (Schlieper et al 1981). As charge density depends on the pH of the bathing solution and on the pK value of the drug, we made comparative studies of the effects of drugs having secondary and tertiary amino groups and possessing pK values between 2·5 and 9·4 on cardiac tissues at pH 7·4 and 9.

MATERIAL AND METHODS

Materials

(±)-Propranolol HCl was obtained from ICI-Pharma, Plankstadt, FRG; amethocaine (tetracaine HCl), procaine (HCl) and benzocaine (HCl) from Hoechst AG, Frankfurt, FRG; lignocaine (lidocaine HCl) from Astra, Wedel, FRG. All other chemicals were obtained from Merck, Darmstadt, FRG.

Isolated heart preparation

Male guinea-pigs were killed by a blow on the neck. Papillary muscles and left atria were isolated and suspended in organ baths containing 20 mL Krebs-Hensleit solution at 31 °C and aerated with carbogen (95% O_2/5% CO_2). The tissue preparations were fixed on platinum electrodes and stimulated at 1 Hz, 3 ms pulse duration with an intensity twice the threshold value. Contractions were recorded under isometric conditions by inductive force transducers (Statham UC II). Preload was 1 g for atria and 0·5 g for papillary muscles.

For the measurements of functional refractory period (FRP) the first stimulus was followed by a second having the same amplitude and duration. FRP was the time interval after which a second comparable contraction could be induced.

Physicochemical constants

The partition coefficients were determined according to Hellenbrecht et al (1973). For separating the soy bean lipids from the buffer (100 mM KCl, 1 mM glycine) the samples were centrifuged at 55 000 rev min^{-1} (average g = 287 000). The degree of ionization was determined by applying the Henderson-Hasselbalch equation.

Measurements of action potential

The papillary muscles were mounted horizontally in a Perspex chamber which was perfused continuously with Krebs-Henseleit solution at a flow rate of 10 mL min^{-1}, kept at 31 °C and aerated with carbogen. The tissues were stimulated as mentioned before and were allowed to equilibrate for 1 h.

Action potentials from papillary muscles were recorded by conventional glass microelectrodes, filled with 3 mol L^{-1} KCl, having resistances higher than 10 MΩ. The signal of the intracellular electrode was fed into one channel of a dual microprobe system (model KS-7000, WPI) and displayed on storage oscilloscopes (Tektronix 5103 N and Gould OS 4000 with output unit 4001). A second electrode, placed closely to the first but extracellularly, served as a reference and was connected to the second channel of the dual microprobe system. The action potential was differentiated electronically ($\dot{V}_{max}$).

All the signals were displayed on oscilloscopes and on pen recorders for exact evaluation. From these records, action potential duration at 30 and 90% repolarization (APD30 and APD90) was determined. The experiment was started when a stable impalement of the microelectrode was guaranteed.

Drugs were added directly to the superfusing Krebs-Henseleit solution.

The Krebs-Henseleit solution had the following composition (mM): at pH 7·4, 118·0 NaCl, 4·7 KCl, 2·52 $CaCl_2$, 1·19 $MgSO_4$, 1·18 NaH_2PO_4, 24·9 $NaHCO_3$, 11·1 glucose; at pH 9, 143·0 NaCl, 4·7 KCl, 2·52 $CaCl_2$, 1·19 $MgSO_4$, 1·0 $NaHCO_3$, 11·1 glucose.

The solution for pH 9 was adjusted with NaOH, aerated with oxygen and routinely checked with a pH meter; the solution for pH 7·4 was aerated with carbogen.

Benzocaine was dissolved in dimethylsulphoxide (DMSO) and diluted with water (1 : 4). DMSO in the concentrations used had no effect either on contractility or on action potential.

RESULTS

Isolated cardiac tissue preparations

Benzocaine, propranolol, amethocaine, lignocaine and procaine decrease the force of contraction of the atrial and papillary muscle preparations in a concentration-dependent manner. The drug concentrations which caused a 50% decrease in force of contraction (EC50) calculated from concentration-response curves according to Hafner et al (1977) are listed in Table 1. The drug effects on both tissues are higher at pH 9 than at pH 7·4.

The EC50 values on papillary muscles are smaller than those on left atria, indicating a higher sensitivity of the former tissue to the drugs. This effect is seen in Fig. 1, which shows concentration-response curves of benzocaine (as representative of the tested compounds) on isolated cardiac tissues at pH 7·4 and 9.

Table 2 shows the effect of the drugs on the FRP of left atria. Potency is represented by concentrations causing a 50% increase of FRP. All the drugs except benzocaine caused a significant prolongation. Amethocaine had the highest potency followed by propranolol, lignocaine and procaine. The effects are higher at pH 9 than at pH 7·4.

Table 1. The drug concentrations which cause a 50% decrease in force of contraction of guinea-pig left atria and papillary muscles (EC50, μM) at pH 7·4 and 9 as derived from concentration-effect curves (n = 8) (Schlieper 1984).

	Left atria		Papillary muscles	
Drug	pH 7·4	pH 9	pH 7·4	pH 9
Propranolol	18·8 ± 0·9	9·8 ± 0·5	7·7 ± 0·3	3·1 ± 0·1
Amethocaine	16·9 ± 1·1	12·6 ± 0·7	14·2 ± 0·7	3·1 ± 0·2
Lignocaine	256·1 ± 12·1	104·8 ± 6·4	76·2 ± 3·6	56·0 ± 2·8
Benzocaine	257·7 ± 11·4	179·4 ± 8·3	304·1 ± 10·1	162·2 ± 7·4
Procaine	805·3 ± 46·8	1162·1 ± 92·6	518·7 ± 23·2	282·2 ± 16·3

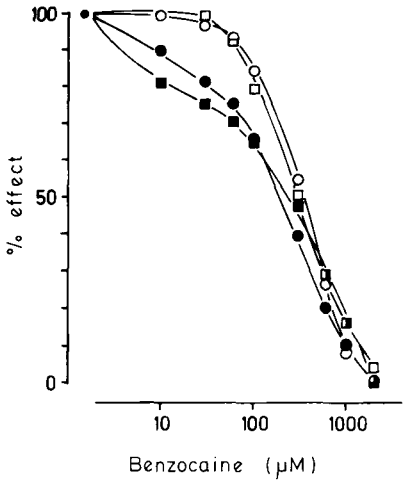

FIG. 1. Concentration-response curves of benzocaine on left guinea pig atria (□ and ■) and papillary muscles (○ and ●) at pH 7·4 (open symbols) and at pH 9 (closed symbols). The force of contraction is expressed as percentage of the control value (% effect), n = 8.

Electrophysiological studies

Fig. 2 shows original registrations of control action potentials, $\dot{V}_{max}$ and force of contraction of the papillary muscles at pH 7·4 and 9. A pH change from 7·4 to 9 caused a broadening of the action potential at 30 and 90% repolarization and a small decrease in $\dot{V}_{max}$. The small increase in inotropy disappeared after 30 min while the action potential parameters remained constant.

Table 2. The drug concentrations which cause a 50% increase in functional refractory period of guinea-pig left atria (EC50, μM) at pH 7·4 and 9.

Drug	pH 7·4	pH 9
Amethocaine	22·6 ± 1·6	14·7 ± 1·4
Propranolol	86·4 ± 6·3	53·6 ± 5·9
Lignocaine	183·7 ± 10·7	81·8 ± 8·9
Procaine	742·9 ± 39·9	593·2 ± 33·8
Benzocaine	0	0

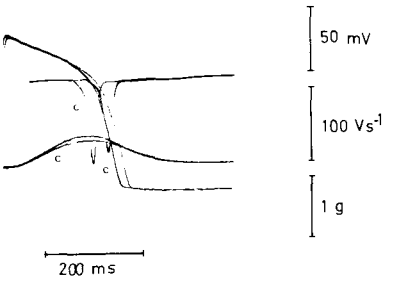

FIG. 2. The action potential, the maximal upstroke velocity ($\dot{V}_{max}$) and force of contraction as recorded from one cell of the guinea-pig papillary muscle at pH 7·4 and after changing to pH 9 (C = pH 7·4).

Fig. 3A–E summarizes the drug effects on action potentials, $\dot{V}_{max}$ and forces of contraction at pH 7·4 and 9. The resting membrane potential stayed constant throughout all the experiments and was not changed by any of the drugs. Changes in action potential and $\dot{V}_{max}$ by the drugs were the same at pH 7·4 and 9 with effects being more pronounced at pH 9. Propranolol, lignocaine and benzocaine decreased the action potential duration during the whole phase of repolarization, while amethocaine and procaine decreased its duration at 30% repolarization but increased it at 90% repolarization. Action potential amplitude and V_{max} were decreased by all the drugs, except by benzocaine, which even at 600 μM caused no observable changes in action potential amplitude or $\dot{V}_{max}$. The drug-induced decrease in force of contraction correlates with the changes of action potential parameters in all experiments; these are summarized in Table 3.

In all experiments the results are the means ± s.e.m. For all given values s.e.m. was <6%.

DISCUSSION

Our comparative studies of drug effects on atrial tissues give several indications that the charged form

Table 3. The effects of different drugs on action potential amplitude, maximum rate of rise ($\dot{V}_{max}$) and action potential duration at 30 and 90% repolarization at pH 7·4 and 9. The drug effects are expressed as percent decrease (−) or increase (+) from control. Average control values are: AP amplitude (mV): pH 7·4: 130·6 ± 1·1, pH 9: 129·8 ± 1·6; $\dot{V}_{max}$ (V/s): pH 7·4: 117·9 ± 11·6, pH 9: 111·4 ± 12·5. Control APD values varied significantly, depending on the site of impalement, n = 3.

Drug	Concn μM	AP ampl. at pH 7·4	AP ampl. at pH 9	$\dot{V}_{max}$ at pH 7·4	$\dot{V}_{max}$ at pH 9	APD30 at pH 7·4	APD30 at pH 9	APD90 at pH 7·4	APD90 at pH 9
Propranolol	14	−3	−5	−23	−30	−14	−18	−8	−13
Amethocaine	15	−6	−7	−42	−51	−3	−4	0	+2
Lignocaine	100	−1	−7	−1	−42	−12	−9	−7	−5
Procaine	700	−6	−8	−27	−52	−21	0	−8	+17
Benzocaine	300	0	0	0	0	−10	−11	−10	−10

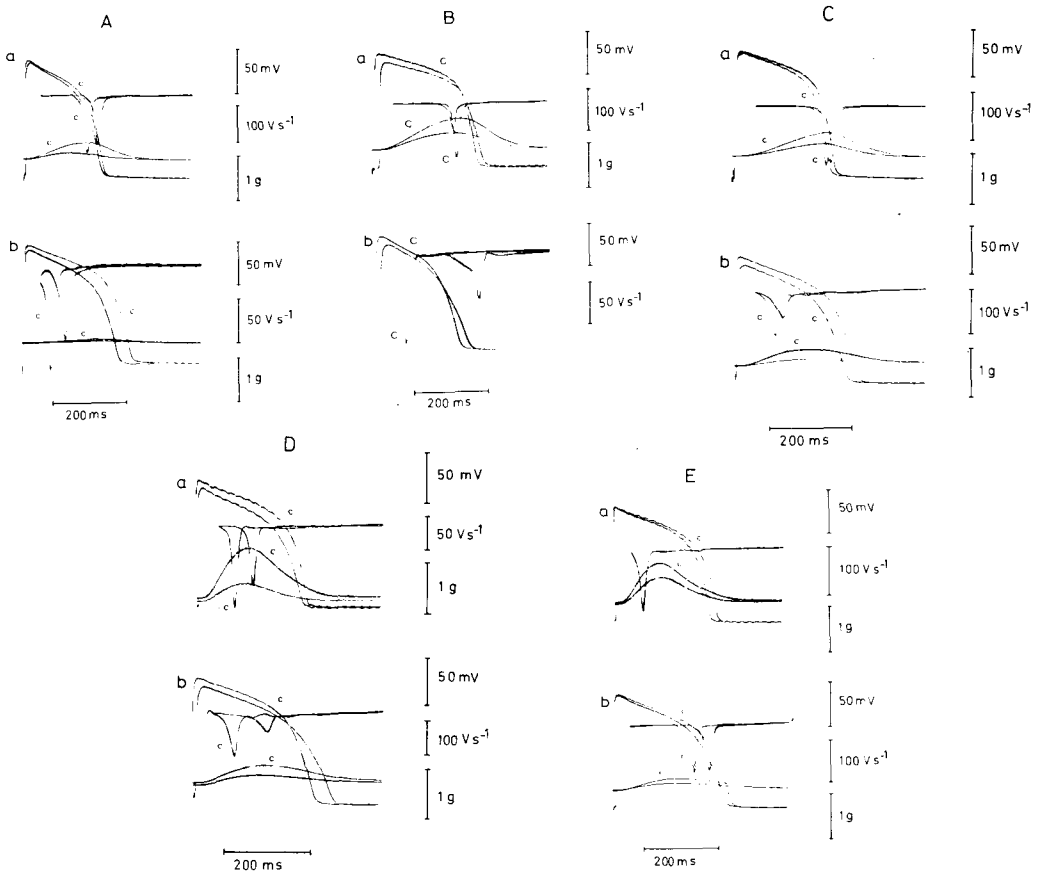

FIG. 3A–E. Effects of different drugs on action potentials, V_{max} and force of contraction of papillary muscles at pH 7·4 (A) and 9 (B). C = control. (A) a: 14 μM propranolol, 20 min, pH 7·4. B: 14 μM propranolol, 13 min, pH 9. (B) a: 15 μM amethocaine, 20 min, pH 7·4. b: 30 μM amethocaine, 8 min, pH 9. (C) a: 100 μM lignocaine, 15 min, pH 7·4. B: 100 μM lignocaine, 10 min, pH 9. (D) a: 700 μM procaine, 10 min, pH 7·4. B: 700 μM procaine, 10 min, pH 9. (E) a: 150 μM benzocaine, 10 min, pH 7·4. b: 150 μM benzocaine, 10 min, pH 9.

of the drug is responsible for certain biological effects. Benzocaine for example, uncharged at pH 7·4 and 9, had no effect on action potential amplitude, $\dot{V}_{max}$ or FRP. The other drugs, being charged secondary and tertiary amines, showed clear effects on these three parameters.

When they are incorporated into liposomes, secondary and tertiary amines change the zeta potential (the potential at the hydrodynamic plane of shear, which is the border between fixed surface charges and the surrounding electrolyte). The relation between zeta potential and fixed surface charges is given by the Gouy-Chapman theory (Schlieper et al 1981). The drugs are incorporated into the phospholipid membrane by hydrophobic interaction in such a way that the protonated positively charged amino group is located near the negatively charged phosphate group of the phospholipids (Schlieper & Michaelis 1983). The negative surface charge of the liposomes will therefore be neutralized and the surface may even become positively charged (Schlieper & Steiner 1983). A similar mechanism may hold for the biological cell membrane, where the positively charged amino group is orientated towards the extracellular space. The increase in positive surface charges causes a reduction in fast sodium current by ionic repulsive forces. According to this hypothesis, smaller effects would be expected at pH 9 from drugs with pK values smaller than 9 because a high percentage of such a drug would be in the uncharged form. However, amethocaine and lignocaine with pK values of 8·48 and 7·97, respectively, showed stronger cardiac effects at pH 9 than at pH 7·4. This contradictory finding is explained with the results summarized in Table 4. The values show that even at pH 9 the surface potential of the

Table 4. Changes in zeta potential ($\Delta\zeta$, mV) of soy bean liposomes caused by the drugs, their lipid partition coefficients (P) and the percentage of ionized drug (I) at pH 7 and 9. The zeta potential values are taken from the linear part of the effect-concentration curves (from 0·1 to 1 mM drug concentration), quoted from Fig. 4 in Schlieper et al (1981).

	pH 7·0			pH 9·0			
Drug	$\Delta\zeta$	P	I	$\Delta\zeta$	P	I	pK
Propranolol	59·4	316·1	99·6	67·5	413·7	72·0	9·41
Amethocaine	45·6	235·9	96·8	52·7	314·4	23·2	8·48
Lignocaine	14·2	*	90·3	19·0	*	8·5	7·97
Procaine	9·5	40·8	99·1	16·2	51·3	51·1	9·02
Benzocaine	0	84·1	0	0	89·3	0	2·50

* Not determined.

liposomes is drastically changed by those drugs which are mainly in their uncharged forms. Although the amount of charged drug in the buffer medium is decreased at pH 9, a considerable amount of the drug located in the liposome surface (even more than at pH 7), is in its charged form, as shown by changes in zeta potential ($\Delta\zeta$ values). At pH 9 the drug partitions to a greater extent into the lipid phase (Table 4). The lipid/buffer partition coefficients (P) correlate with the EC50 values of the drugs. Propranolol has the highest P value, i.e. the highest affinity for the lipids and the lowest EC50 value, i.e. the highest potency on isolated cardiac tissue. The drugs may be ranked based on the lipid/buffer partition coefficients and negative inotropic activities: propranolol > amethocaine > benzocaine > procaine.

The changes in $\dot{V}_{max}$ by the various drugs did not correlate with P or EC50. This indicates that the mechanisms for action potential upstroke are mostly independent of the action on the force of contraction of cardiac tissues.

Most of the drugs changed the functional refractory period of the papillary muscles, amethocaine being the most effective, followed by lignocaine and procaine. Uncharged benzocaine, even at concentrations up to 1 mM showed no effect on $\dot{V}_{max}$ and functional refractory period of the cardiac tissues, suggesting that only charged drugs influence those parameters.

Changes in functional refractory period originate firstly from changes in the fast repolarization, governed by K^+ efflux and secondly from changes in the recovery of the Na^+- and Ca^{2+}-system (Coraboeuf 1978). Gettes & Reuter (1974) determined the time constant of the recovery of the Na^+- and Ca^{2+}-system as a function of membrane potential and found that the recovery increased with increasing resting potential. In our experiments we did not observe any change in resting membrane potential by the different drugs. But we postulate a change in transmembrane potential originating from the more positively charged outer membrane surface. This could increase the recovery and could be the reason for the increase in refractory period and the other characteristic changes in the action potential. A change in transmembrane potential should also influence the threshold of excitation, as we observed with the charged drugs but not with benzocaine. Amethocaine and procaine caused a prolongation of the action potential at 90% repolarization, which could be interpreted as a delay of the repolarizing potassium current. This interpretation is in agreement with the results of Almers (1976) who reported a delay in potassium currents in frog skeletal muscle under the influence of amethocaine.

Besides having a tertiary amino group, amethocaine and procaine also have an amino group in the para position to the carbonyl group and this group might be responsible for the common effect of both drugs on repolarizing potassium currents.

Whether the force of contraction, $\dot{V}_{max}$ or functional refractory period are first influenced by the drug-induced changes in charge density cannot be derived from our experiments.

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