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A comparison of the electrophysiological actions of phentolamine with those of some other antiarrhythmic drugs on tissues isolated from the rat heart

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1 Glass microelectrodes were used to record transmembrane electrical activity from cells located just beneath the endocardial surface of segments from the atrial and right ventricular free walls of rat hearts during superfusion and electrical stimulation *in vitro* at 37°C.

2 Availability of the fast sodium channels for current flow was inferred from the maximum rate of rise of membrane potential during phase 0 of the action potential.

3 Phentolamine mesylate (2 to 20 μM) caused a concentration-dependent block of the fast sodium channel. This was reflected in prolongation of the refractory period and slowing of recovery of excitability following the action potential, without significant change in action potential duration or resting membrane potential.

4 Increase in the concentration of KCl in the superfusate from 5 to 10 mM depolarized the muscle and potentiated the blocking action of phentolamine. Both the depolarizing and the phentolamine-potentiating actions of KCl were counteracted by simultaneous elevation of the concentration of $CaCl_2$ in the superfusate from 2 to 10 mM.

5 The blocking action of phentolamine was enhanced by increasing the frequency of electrical stimulation in the range 0.01 to 10 Hz.

6 In respect of the properties listed above, lignocaine hydrochloride was similar to phentolamine but was different from quinidine sulphate in that the effects of the latter drug were not potentiated by KCl.

7 Two other α-adrenoceptor antagonists, prazosin and yohimbine, both displayed actions similar to those shown by phentolamine. Tolazoline was only weakly active and dihydroergotamine (60 μM) was inactive. Dibenamine and phenoxybenzamine, unlike the previously named drugs, caused an irreversible block of the fast sodium channel. These blocking actions of α-adrenoceptor antagonists were not prevented by simultaneous exposure to the α-adrenoceptor agonist phenylephrine (1 mM).

8 Muscle from both reserpine pretreated and non-pretreated rats responded indistinguishably to phentolamine.

Introduction

Phentolamine has been known to possess an antiarrhythmic action on the heart for more than thirty years (Leimdorfer, 1952). This has been considered by many to be a laboratory curiosity and the drug has been little utilized for therapeutic purposes, despite enthusiastic claims by several groups of cardiologists. Clinical experience with the drug has been reviewed by Gould & Reddy (1976). Interest in the antiarrhythmic action of phentolamine was revived recently, by reports that phentolamine, in company with certain other α-adrenoceptor antagonists, supresses ventricular tachyarrhythmias produced in experimental animals by coronary artery ligation and reperfusion (Sheridan, Penkoski & Sobel, 1980; Stewart, Burmeister, Burmeister & Lucchesi, 1980; Penny & Sheridan, 1982; Pogwizd, Sharma & Corr, 1982). These experimental findings agree with the results of an earlier study in patients with acute myocardial infarction where phentolamine suppressed ventricular premature beats (Gould, Reddy, Weinstein & Gomprecht, 1975). It is significant that, in several studies, α-adrenoceptor antagonists were antiarrhythmic in circumstances where β-adrenoceptor antagonists were inactive. Unlike most other antiar-

rhythmic drugs, which tend to reduce cardiac output and aggravate existing cardiac failure, phentolamine increases cardiac output and may be used to relieve existing cardiac failure (Kelly, Delgado, Taylor, Pitt & Ross, 1973; Walinski, Chatterjee, Forrester, Parmley & Swan, 1974; Perret, Gardaz, Reynaert, Grimbert & Enrico, 1975).

The pharmacological mechanisms by which phentolamine exerts its antiarrhythmic action are still uncertain. Rosen, Gelband & Hoffman (1971) showed that the drug blocks the fast sodium channel in cardiac cell membranes in a manner similar to that displayed by lignocaine and quinidine: a class I action in the nomenclature of Vaughan Williams (1974). It is unclear, however, to what extent this electrical property is dependent upon α-adrenoceptors. Stewart *et al.* (1980) concluded that α-adrenoceptors were not involved in the blockade of the fast sodium channel, but Williams, Griffith & Albrecht (1978) reached an opposite conclusion. Corr, Shayman, Kramer & Kipnis (1981) not only invoked α-adrenoceptors in the antiarrhythmic action of phentolamine but ascribed its effectiveness in ischaemic hearts to an increased number of α-adrenoceptors in ischaemic compared with normal myocardium. The experiments described in the present paper examine the actions of a range of α-adrenoceptor agonists and antagonists in order to shed some light upon this controversy.

Methods

Male albino rats of the Sprague Dawley strain weighing 320–350 g were killed by a blow to the head. Hearts were excised rapidly and placed, unless otherwise stated, in a solution of the following composition (mM): NaCl 138, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, NaH_2PO_4 0.5, $NaHCO_3$ 10 and glucose 10 and gassed with a mixture of 95% O_2 plus 5% CO_2, giving a pH of 7.2. Strips of muscle measuring approximately 3 by 8 mm were prepared from the left or right atrial or the right ventricular free walls. The strips were attached, endocardial face upwards, to the base of a superfusion trough maintained at 37°C. Glass microelectrodes filled with a 3 M solution of KCl and having an electrical resistance of $1.0-1.5 \times 10^7$ ohms were used to record membrane potentials (V_m) from muscle cells situated just beneath the endocardial surface. These voltages were transmitted via a cathode-follower circuit to a dual channel oscilloscope equipped with a camera, the second channel of which displayed a time-differentiated derivative of the V_m signal. The differentiator was calibrated using a sawtooth waveform generator, The peak value of the differentiated V_m signal ($\dot{V}_{max}$) during phase 0 of the action potential provides a convenient measure of the availability of the fast sodium channel for current flow, hereafter termed the excitability of the membrane (Gettes & Reuter, 1974). The muscle was stimulated via a pair of platinum wires with square wave pulses, each of 10 V and 1 ms duration, at a rate between 0.05 and 10 Hz, but at 1 Hz unless specified otherwise in the text. Facilities were available not only to stimulate the muscle with single pulses but also, once every 20 s, to provide a pair of stimuli, the members of which could be separated from each other by any chosen interval between 5 and 1000 ms. In this way it was possible to determine the excitability of the membrane during the partially refractory period caused by a preceding action potential and hence to study the kinetics of recovery of excitability.

Drugs were added to the bathing fluid as freshly prepared concentrated solutions. Unless otherwise specified the tissue was exposed to a drug for 30 min prior to electrical measurement. The following drugs were employed: phentolamine mesylate (Ciba Geigy); prazosin hydrochloride (Pfizer); yohimbine hydrochloride (Koch-Light); dibenamine hydrochloride (Butler); phenoxybenzamine hydrochloride (Smith, Kline and French); lignocaine hydrochloride (Sigma); quinidine sulphate (Sigma); dihydroergotamine tartrate (Sigma); tolazoline hydrochloride (Ciba Geigy); (−)-phenylephrine hydrochloride (Koch-Light); adrenaline tartrate (B.D.H.).

Results

Effects of α-adrenoceptor antagonists on ventricular muscle

Ventricular muscle stimulated with single pulses at 1 Hz was exposed for 30 min to the standard bathing fluid to which phentolamine was added at final concentrations of 1 to 20 μM. Figure 1 shows that phentolamine produced a concentration-dependent decrease in $\dot{V}_{max}$ without causing a significant change in diastolic V_m. In this concentration range the drug prolonged the refractory period of muscle subjected to paired stimulation with only small and statistically insignificant changes in the duration of the action potential measured to the point of 95% repolarization (APD), as shown in Figure 1. Using paired stimuli, and in the absence of drugs, the muscle first became re-excitable to electrical stimulation when the membrane potential became more negative than −67 mV, which represents 80% of full repolarization. In the presence of a concentration of phentolamine of 3 μM or greater, the muscle needed to have become more than 95% repolarized before it became re-excitable.

The kinetics of recovery of excitability during paired stimulation were studied by plotting $\dot{V}_{max}$

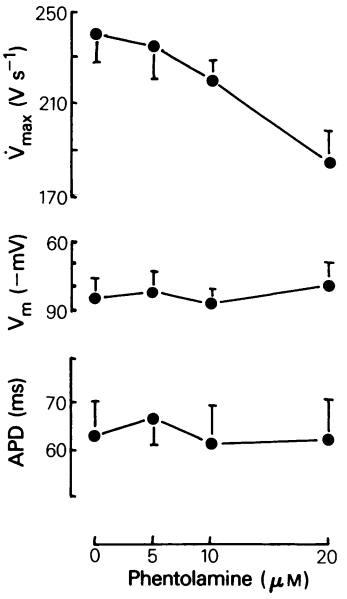

Figure 1 Effect of exposure of ventricular muscle to various concentrations of phentolamine on the maximum rate of phase 0 depolarization ($\dot{V}_{max}$), diastolic membrane potential (V_m) and action potential duration measured to the point of 95% repolarization (APD) during stimulation at 1 Hz. Vertical bars indicate s.e. Each point is the mean of between 24 and 58 measurements.

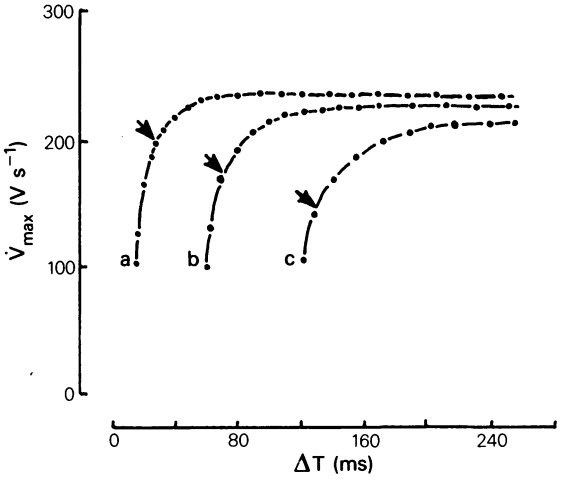

Figure 2 Responses of ventricular muscle to paired stimuli. The second stimulus of a pair was timed to produce an action potential during phase 3 of the action potential caused by the first member of each pair. The $\dot{V}_{max}$ values were obtained from variously timed second responses in the absence of drug (a), and in the presence of phentolamine at 5 μM (b) and at 10 μM (c). Arrows indicate the time constants for each curve.

values for the second action potential against the time interval (ΔT) between the point of attainment of 80% repolarization during the first action potential and the start of phase 0 of the second action potential, as shown in Figure 2. The relationships obtained in these plots were approximately exponential, thus permitting the calculation of a time constant (TC) for recovery of excitability. Phentolamine caused a concentration-dependent slowing of recovery of excitability. The concentration needed to double the value of TC measured in the absence of drug was calculated to be 3.5 μM. This is referred to as the TC2 concentration. Note that ΔT was measured in all cases from the point of 80% repolarization, irrespective of whether or not the tissue had actually become re-excitable by this time.

The concentration of KCl in the bathing fluid is well known to affect the electrical properties of cardiac muscle. It was of interest, therefore, to determine the extent to which the ability of phentolamine to block the fast sodium channel was influenced by the prevailing concentration of KCl. Raising the concentration of KCl above that in the standard bathing fluid caused diastolic depolarization, and lowering it had the opposite effect. Muscle depolarized by exposure to 10 mM KCl showed a TC for recovery of excitability which was nearly twice that of muscle exposed to the standard bathing fluid (Table 1). Conversely, muscle hyperpolarized by exposure to 2.5 mM KCl had a TC which was only about half of that measured at the normal KCl concentration of 5 mM (Table 1). In the hyperpolarized state the ability of 3.5 μM phentolamine to block the fast sodium channel was reduced, and in the depolarized state it was enhanced (Table 1). The concentration of phentolamine chosen for this comparison (3.5 μM) was the TC2 concentration measured in 5 mM KCl (Table 1). A measure of the sensitivity of the muscle to the blocking action of phentolamine was obtained by expressing the TC found in the presence of 3.5 μM phentolamine as a multiple of that found in the absence of the drug. In a bathing fluid containing 10 mM KCl, phentolamine caused a 2.9 fold prolongation of TC, which contrasts with a figure of 1.3 obtained at a KCl concentration of 2.5 mM. It was also of interest to determine the extent to which the phentolamine-potentiating action of KCl was influenced by the prevailing concentration of $CaCl_2$. Figure 3 shows that raising the concentration of $CaCl_2$ counteracted the prolongation of TC and the phentolamine-potentiating effect of an elevated KCl concentration. Similarly, the simultaneous lowering of the concentrations of both KCl and $CaCl_2$ counteracted both the shortening of TC and the phentolamine-opposing effects seen when only the KCl concentration was lowered (Figure 3).

The ability of several class I antiarrhythmic agents

Table 1 Effects of treating rat ventricular muscle with various drugs

Drug	(μM)	V_m (−mV)	APD (ms)	RP (ms)	TC (ms) measured at a KCl conc of 5 mM	10 mM	2.5 mM	TC ratio†
Control	–	84± 9	63±7	34±12	30± 9	55±14	15± 4	4
Phentolamine	3.5	83± 8	65± 6	72±14*	59±13*	160±23*	20±05	8
Phentolamine	10	86± 6	61± 9	154±18*	130±20*	280±29*	28± 7	10
Phenylephrine	1000	91± 5	59± 8	37±10	33± 8	52±12	15± 3	3
Adrenaline	1000	80± 8	58± 5	39± 9	28± 7	56±15	13± 5	4
Phentolamine plus Phenylephrine	10 1000	82± 6	62± 9	144±22*	138±24*	269±24*	30± 6*	9
Prazosin	1	78± 7	66± 8	80±11*	61±10*	173±19*	18± 4	10
Prazosin	2	83± 5	70± 7	166±24*	150±25*	291±26*	34± 8*	9
Yohimbine	4	84±10	73± 7	76±13*	66±12*	181±20*	29± 7*	6
Yohimbine	8	88± 9	60± 6	140±25*	129±20*	214±22*	35± 8*	6
Phenoxybenzamine	5	76± 9	65±10	68±10*	57± 9*	149±18*	30± 7*	5
Phenoxybenzamine	10	77± 7	67± 8	160±19*	119±27*	247±30*	40± 7	6
Dibenamine	5	81± 6	64± 5	61±12*	55± 8*	161±17*	16± 5	10
Dibenamine	10	90± 7	68± 8	141±26*	110±19*	202±25*	27± 6*	8
Dihydroergotamine	60	84± 8	67± 7	36± 8	32± 7	48±11	17± 6	3
Tolazoline	1000	85± 9	57± 9	85±20*	71±13*	185±21*	24± 5	8
Lignocaine	10	79±10	66± 8	72±18*	68±14*	151±16*	22± 5	7
Lignocaine	20	89± 8	68± 8	132±17*	155±22*	306±27*	33± 8*	9
Quinidine	8	92± 7	69± 7	70± 8*	65±10*	88±14*	47± 9*	2
Quinidine	16	85± 6	89± 6	151±23*	141±28*	148±16*	68±10*	2

Diastolic membrane potential (V_m), action potential duration measured to the point of 95% repolarization (APD), refractory period (RP) and the time constant (TC) for recovery of excitability were measured as described in the text using the standard bathing fluid. In addition, TC values were determined at KCl concentrations of 10 and 2.5 mM respectively. †The TC ratio represents the ratio of the TC values measured at these latter concentrations. Tabulated values represent the means of 12 to 80 measurements ± s.e.
*indicates that a statistically significant difference exists (Students t test, $P<0.05$) between a mean and its non-drug-treated control.

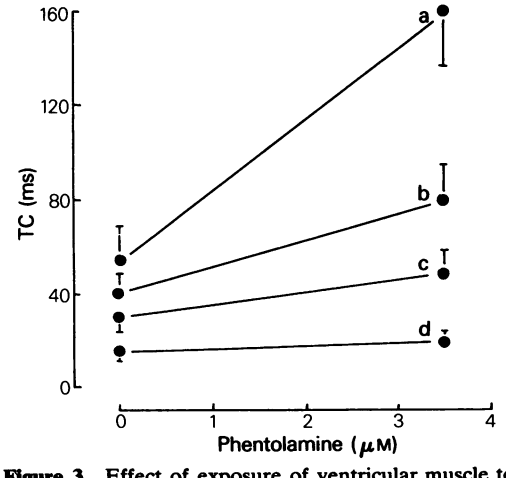

Figure 3 Effect of exposure of ventricular muscle to 3.5 μM phentolamine on the time constant (TC) for recovery of excitability during paired stimulation in the presence of various concentrations of KCl (10 mM in a and b; 2.5 mM in c and d) and of $CaCl_2$ (10 mM in b; 2 mM in a and d; 0.4 mM in c); vertical bars indicate s.e. Each point is the mean of between 12 and 16 measurements.

to block the fast sodium channel in heart muscle is known to depend upon the frequency of stimulation. Figure 4 shows that when ventricular muscle was stimulated in the present experiments at 0.1 Hz or less, 5 μM phentolamine was ineffective in blocking the fast sodium channel, whereas at 10 μM the drug was effective at a stimulation frequency of 0.1 Hz, although not at 0.01 Hz.

The ability of phentolamine to block fast sodium channels raised the question of whether or not other α-adrenoceptor antagonists shared this electrical property. A variety of α-adrenoceptor antagonists, therefore, were tested. Table 1 shows their effects on the refractory period (RP) and the TC for recovery of excitability. Both yohimbine and prazosin blocked the fast sodium channel and delayed the recovery of excitability; actions which were potentiated by an elevated concentration of KCl. The action of these α-adrenoceptor antagonists was reversed by washing with drug-free bathing fluid for 10–60 min. Higher concentrations of some of these drugs had a more persistent action than the lower concentrations, but at TC2 concentrations and below, all were reversed fully by the end of an hour. In contrast, the inhibitory

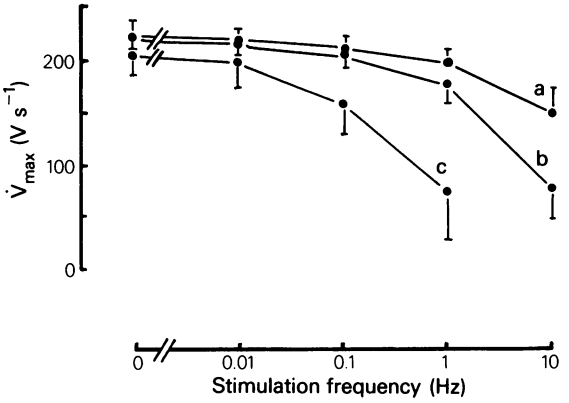

Figure 4 Effect of varying rates of stimulation on the maximum rates of phase 0 depolarization ($\dot{V}_{max}$) shown by ventricular muscle in the absence of drug (a) and in the presence of phentolamine at 5 μM (b) and 10 μM (c). Vertical bars indicate s.e. Each point is the mean of between 24 and 58 measurements.

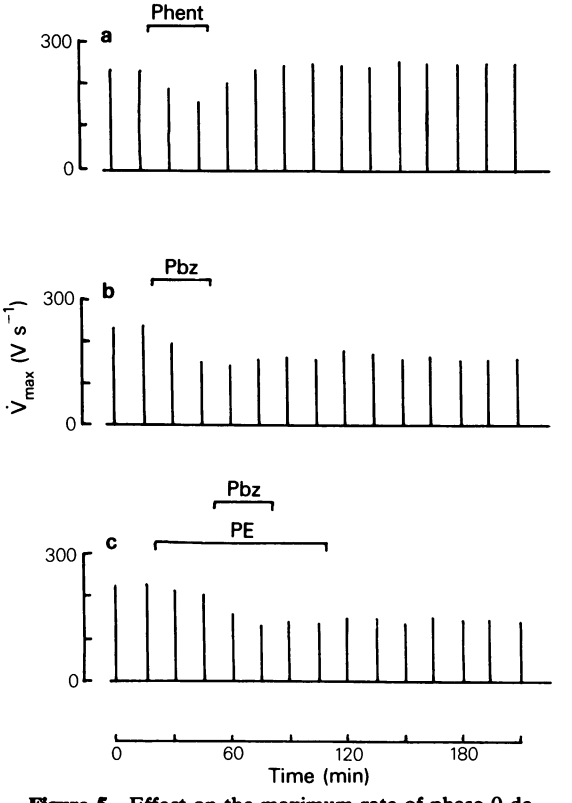

Figure 5 Effect on the maximum rate of phase 0 depolarization ($\dot{V}_{max}$) shown by ventricular muscle during stimulation at 1 Hz while being exposed to 15 μM phentolamine (Phent), 20 μM phenoxybenzamine (Pbz) and 1 mM phenylephrine (PE) during the periods indicated by the horizontal bars.

actions of both phenoxybenzamine and dibenamine were not detectably reversible within 2 h, as shown in Figure 5. The only recognized α-adrenoceptor antagonist from among those tested which failed to block the fast sodium channel significantly was dihydroergotamine, even at a concentration of 60 μM. Tolazoline, however, was only weakly active.

The blocking action of α-adrenoceptor antagonists on the fast sodium channel raised the question of whether or not α-adrenoceptor agonists would show any effect. Despite being tested at concentrations of up to 1 mM, however, phenylephrine not only failed to exert a measurable effect on its own (Table 1) but also failed to modify the blocking action of phentolamine and phenoxybenzamine (Figure 5). Adrenaline was similarly inert (Table 1).

Some of the actions of phentolamine on the heart are known to depend upon the tissue stores of catecholamines. This raised the question of whether or not the ability of phetolamine to block the fast sodium channel was similarly dependent. To investigate this possibility rats were pretreated with reserpine to deplete them of their myocardial stores of catecholamines. Reserpine was administered intraperitonealy in a dose of 1 mg per day for the 2 days before the rats were killed. Ventricular muscle from reserpine-treated animals responded to phentolamine in exactly the same way as muscle from animals with intact catecholamine stores.

Effects of lignocaine and quinidine on ventricular muscle

Table 1 shows that phentolamine, lignocaine and quinidine had similar actions on the fast sodium channel in the present experiments. The only significant difference between their actions was in respect of the ability to be potentiated by elevated concentrations of KCl. The actions of both lignocaine and phentolamine were much greater at 10 than at 2.5 mM KCl, whereas the actions of quinidine were little changed.

Effects of phentolamine on atrial muscle

The action of phentolamine on left atrial muscle was similar to its action on ventricular muscle. The drug caused a dose-dependent decrease in $\dot{V}_{max}$, a prolongation of the refractory period and a slowing of the restoration of excitability after an action potential. The left atrium was slightly, but not significantly, more sensitive than the ventricle to this drug. Experiments with the right atrium were more difficult to conduct on account of the high inherent rhythmicity of this chamber, and pacing rates of 4 Hz were required for most experiments in which a fixed rate of response was needed. Even this was insufficient at

low concentrations of KCl or high concentrations of $CaCl_2$. In the standard bathing fluid, and at a pacing frequency of 4 Hz, phentolamine was shown to decrease $\dot{V}_{max}$ without producing a significant change in the diastolic value of V_m or the APD. The TC2 concentration was 5.5 μM, indicating that the right atrium was less sensitive to this drug than the other parts of the heart which were studied. At concentrations of 10 μM and higher the inherent rhythmicity of the right atrium was inhibited by phentolamine.

Discussion

The present experiments have shown that phentolamine decreases $\dot{V}_{max}$ of cardiac action potenconfirming earlier findings (Ledda & Marchetti, 1971; Rosen *et al.*, 1971; Sada, 1978). This action has usually been taken as a sign of blockade of the fast sodium channels. Very similar properties were displayed by lignocaine in both the present experiments and those of previous workers (Chen, Gettes & Katzung, 1975).

The ability of phentolamine to block the fast sodium channels in the present experiments depended upon the frequency with which the muscle was stimulated, confirming earlier observations of Sada (1978). In this respect the action of phentolamine resembles that of lignocaine and quinidine described by previous workers (Johnson & McKinnon, 1957; Heistracher, 1971; Weld, Coromilas, Rottman & Bigger, 1982). Dependence upon stimulation rate has been attributed by some previous investigators to dissociation of the drug from the ion channel while it is in the resting form (Hondeghem & Katzung, 1977). This hypothesis predicts that the longer the interval between stimuli the less drug will still be bound to the channel when the tissue is next stimulated, and the less block will be produced. This concept has been termed the 'modulated receptor' theory of action.

The blocking action of phentolamine in the present experiments was dependent upon the concentration of KCl in the bathing fluid, which confirms the results obtained previously by Rosen *et al.* (1971), although they did not comment upon the feature. Nevertheless, Figure 5 of their paper shows that phentolamine was approximately three time more potent when the concentration of KCl was raised sufficiently to give a diastolic V_m of −80 mV compared with its potency at a KCl concentration sufficiently low to provide a diastolic V_m of −90 mV. In this respect the actions of phentolamine resemble those of lignocaine demonstrated in both the present experiments and those of previous workers (Chen *et al.*, 1975). The effects of a raised concentration of KCl can be fully accounted for in terms of decline in V_m produced. In the present experiments the electrical responses to an elevated concentration of KCl could be counteracted by an increased concentration of $CaCl_2$ in the bathing fluid. Moreover, elevation of the $CaCl_2$ concentration opposed the ability of a raised KCl concentration to potentiate the actions of both lignocaine and phentolamine. Similar influences of potassium and calcium ions on the electrical responses of the heart to procaine were reported by Bein (1948).

Quinidine shared several of the properties of lignocaine in the present experiments, although certain differences between their actions were noted also. In particular, variation in the concentration of KCl in the bathing fluid failed to modify the blocking action of quinidine, and in contrast to that of lignocaine. Previous workers have reported that the action of quinidine was less dependent than that of lignocaine on the prevailing value of the diastolic V_m (Chen *et al.*, 1975). Extracellular concentrations of KCl in ischaemic myocardium *in vivo* are known to rise to between 10 and 13 mM within a few minutes of the onset of injury (Wiegand, Guggi, Meesman, Kessler & Greitschus, 1979; Hill & Gettes, 1980; Hirche, Franz, Bos, Bissig, Lang & Schramm, 1980). An extracellular concentration of KCl of this order in the present experiments potentiated the actions of both lignocaine and phentolamine, but not those of quinidine. This suggests that quinidine, unlike the other two agents, would not be expected to depress excitability selectively in ischaemically damaged regions of the myocardium. This difference may have therapeutic implications, since the ability of lignocaine, for example, to suppress ventricular arrhythmias in acutely infarcting hearts has been attributed to the preferential blockade of action potentials in ischaemically depolarized regions at the margin of the already dead tissue (El-Sherif, Scherlag, Lazzara & Hope, 1977; Hondeghem & Cotner, 1978; Lazzara, Hope, El-Sherif & Scherlag, 1978; Wang, James & Maxwell, 1979; Wald, Waxman & Downer, 1980; Cardinal, Janse, Eeden, Werner, D'Alnoncourt & Durrer, 1981). The results of the present experiments lead one to expect, therefore, that a similar lignocaine-like selectivity for ischaemically depolarized regions of the myocardium would be displayed by phentolamine. Indeed, Corr *et al.* (1981) demonstrated selectivity for ischaemic myocardium with this drug. They attributed this selectivity, however, to an increased number of α-adrenoceptors in the ischaemic muscle.

Several drugs of diverse chemical structure that share an α-adrenoceptor antagonistic action also share the ability to block the fast sodium channel. This was true in the present experiments of prazosin, a reputedly selective α_1-adrenoceptor antagonist (Frelin, Vigne & Lazdunski, 1982), and of yohimbine, which is claimed to be selective for α_2-adrenoceptors. In contrast to the brief and readily

reversible actions of these two drugs, however, the blocking action of dibenamine and phenoxybenzamine on the fast sodium channel was not reversed by a return to drug-free bathing fluid in the present experiments. Both of these two β-haloalkylamines are considered to combine irreversibly with both β-adrenoceptors and histamine receptors (Goodman & Gilman, 1955). The persistence of effect of the β-haloalkylamines on the fast sodium channels in the present experiments, therefore, constitutes no evidence for the involvement of α-adrenoceptors. Indeed, for two reasons, α-adrenoceptors are probably not involved. Firstly, not all the classical α-adrenoceptor antagonists which were tested shared a blocking action on the fast sodium channel. Dihydroergotamine, a potent α-adrenoceptor antagonist, albeit with partial agonist properties (Chu & Stuermer, 1973; Aellig, 1974), was inactive in the present experiments. It is probably significant also that dihydroergotamine was much less effective than phentolamine in suppressing ventricular tachyarrhythmias produced in dogs by coronary artery ligation (Harris & Bisteni, 1955). A second reason for discounting the involvement of α-adrenoceptors in the results of the present experiments is that phenylephrine and adrenaline, both potent α-adrenoceptor agonists, failed to show any effect opon the fast sodium channel in the present experiments, either when applied on their own or when applied with an α-adrenoceptor antagonist. This is in contrast to previous reports based upon myocardium from species other than the rat where a prolongation of refractory period, albeit sometimes only small, has been demonstrated (Govier, Mosal, Whittington & Broom, 1966; Govier, 1967; Ledda, Marchetti & Manni, 1971). The rat myocardial action potential differs from that of most other species in having little or no plateau (phase 2). It is clear from the work of Ledda, Marchetti & Manni (1971) that the ability of phenylephrine to prolong the refractory period of the ovine myocardium, for example, can be fully accounted for by the prolongation of the action potential during phase 2. The lack of phase 2 in the rat probably accounts for its lack of response to phenylephrine. This simplicity in the rat cardiac action potential renders it more convenient than that of most other species for a study of the effect of fast sodium channel blockers upon the refractory period. Where action potentials having a pronounced plateau are studied, the effects of a drug such as phentolamine, which causes both fast sodium channel block and a shortening of the plateau, will be variable on the refractory period, depending upon which effect predominates. This probably accounts for the variable results which have been reported previously (Ledda & Marchetti, 1971; Rosen *et al.*, 1971; Sada, 1978).

In conclusion, therefore, α-adrenoceptors, at least of the classical type, do not appear to be involved in the lignocaine-like action of the drugs studied in the present experiments. Hondeghem & Katzung (1977) proposed that the receptors with which lignocaine combines within the fast sodium channel are 'modulated' by the associated structures within the channel. Perhaps the receptors with which phentolamine combines in the fast sodium channel differ from classical α-adrenoceptors for a similar reason. Classical α-adrenoceptors do occur in the myocardium and are partly responsible for mediation of the positive inotropism caused by catecholamines (Broadley, 1982), but differ in several important respects from the phentolamine binding sites studied in the present experiments. Similarly, myocardial stores of catecholamines do not appear to be involved in the ability of phentolamine to block the fast sodium channel since muscle from reserpine pretreated and non-pretreated rats responded indistinguishably to the drug. This is in contrast to the positive inotropic action of phentolamine *in vivo* which was shown by Hilliard, Bagwell, Daniell & Freeman (1972) to depend upon such stores. Similar positive inotropism was reported by Benfey (1961) using phenoxybenzamine and by Benfey & Varma (1961) using dibenamine.

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