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Effects of lidocaine, procaine, procainamide and quinidine on electrophysiological properties of cultured embryonic chick hearts

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1 The effects of lidocaine, procaine, procainamide and quinidine were studied on organ-cultured embryonic chick (2–3 day-old) ventricular cells.

2 Lidocaine (10^{-5}–10^{-4} M), in a dose-dependent manner, reduced the rate of pacemaker discharge, the action potential amplitude (APA), the maximum rate of rise (V_{max}) of the upstroke of the action potential and the action potential duration at 50% repolarization (APD_{50}). These changes occurred without alterations in the maximum diastolic potential (MDP). Extracellular electrical field stimulation could still evoke action potentials in cells arrested by 10^{-4} M lidocaine, but 10^{-3} M lidocaine completely abolished electrical activity.

3 Procaine, procainamide and quinidine, at 5×10^{-5} M to 10^{-3} M, depolarized the cells to around -30 mV and reduced APA and V_{max}. Procaine and procainamide increased APD_{50}, but quinidine shortened it. All the effects described disappeared completely in about 40 min of superfusion with drug-free Tyrode solution.

4 Isoprenaline (5×10^{-7} M) and adrenaline (10^{-6} M) restored spontaneous firing of preparations arrested by any of the antiarrhythmic agents and repolarized ventricular cells depolarized by procaine, procainamide or quinidine. Propranolol (5×10^{-7} M) did not affect the depolarization produced by procaine (5×10^{-4} M), but antagonized its reversal by isoprenaline.

5 In contrast, isoprenaline (10^{-6} M) did not produce recovery of automaticity of preparations arrested by verapamil (10^{-5} M).

6 Histamine (10^{-5} M) or strontium (10 mM) were not able to restore rhythmic activity in cells arrested procaine.

7 Application of long (10–15 s duration) hyperpolarizing currents did not reverse the blocking effect of procaine, procainamide and quinidine.

8 The input resistance increased during the procaine-induced depolarization.

9 It is suggested that the four agents studied block the slow Na^+ channels responsible for the upstroke of the action potential in young chick heart cells. A drug-induced decrease in P_K may occur in those cells arrested at low levels of membrane potential.

Introduction

The agents which have class I antiarrhythmic action on myocardium usually possess local anaesthetic activity on nerves (Singh, 1981; Vaughan Williams, 1981). In both types of tissues, class I antiarrhythmic agents reduce the maximum rate of depolarization (V_{max}) of the action potential, local anaesthetic-antiarrhythmic drugs like quinidine, procainamide, lidocaine, also affect other electrical properties of cardiac membranes. When applied at therapeutic concentrations they: (a) depress spontaneous diastolic depolarization in Purkinje fibres, (b) increase the threshold of excitability and (c) reduce the conduction velocity (Bigger & Hoffman, 1980).

Since the exact mechanisms by which these agents exert their therapeutic effects are not clearly understood, and taking into account that they often affect

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more than one ionic channel in cardiac tissues (Carmeliet, 1984), a considerable amount of work has been devoted recently to the study of their actions in many different conditions and preparations (see review by Hauswirth & Singh, 1978). In this respect, the mechanisms of the arrhythmias have been studied in parallel with those of the antiarrhythmic actions of drugs (Lazzara & Sherlag, 1980; 1984; Singer *et al.*, 1981). *In vitro* models of arrhythmias and disturbances of conduction have also been developed in order to facilitate the understanding of their cellular mechanisms (Cranefield, 1975; Dodge & Cranefield, 1982). Recognition of the importance of slow conduction in the genesis of arrhythmias has emerged recently, and the question of whether class I antiarrhythmics would affect phenomena related to slow conduction has become important.

The early reports on the effects of local anaesthetics on slow action potentials were not in agreement. Some investigators found that local anaesthetics did not affect slow channels (Kohlhardt *et al.*, 1972; Carmeliet & Verdonck, 1974; Brennan *et al.*, 1978; Hashimoto *et al.*, 1979), whereas others found depressant effects (Reiser *et al.*, 1974; Josephson & Sperelakis, 1976). It has been shown recently that lidocaine, at therapeutic concentrations, impairs the conduction of slow responses through canine Purkinje fibres depolarized by high K^+ (Lamanna *et al.*, 1982).

In addition to slow Ca^{2+} or Ca-Na channels, there is another kind of channel found in young embryonic chick hearts. Inward current through these channels underlies the slowly-rising action potentials ($10-40\ V\ s^{-1}$), is insensitive to tetrodotoxin (TTX) and depends on the extracellular Na^+ concentration but not on extracellular Ca^{2+}; therefore, it seems to be carried by a flux of Na^+ ions showing slow kinetics (for review see Sperelakis & Pappano, 1983).

In the present paper, we describe the electrophysiological effects of some local anaesthetic-antiarrhythmic agents, namely procaine, procainamide, lidocaine and quinidine on pacemaker and action potentials of organ-cultured young (2–3 day-old) embryonic chick heart cells, and compare their effects with those described for adult and embryonic single myocardial cells.

Methods

Preparation

Cultured ventricular cells obtained from young (2–3 day-old) embryonic chick hearts were used as previously described by Sperelakis & Shigenobu (1974). In brief, the ventricular part was dissected from the heart tube in sterile culture medium, transferred into Erlenmeyer flasks, and swirled on a gyrotatory shaker at 37°C until used for microelectrode impalements 48–72 h later. The culture medium was prepared by adding foetal calf serum (10%) and penicillin-streptomycin ($50\ u\ ml^{-1}$) to medium-199 (all from Gibco); $NaHCO_3$ (26 mM) was added and the pH was adjusted to 7.3 with NaOH.

For electrophysiological studies, a ventricle cultured for 2–3 days was transferred with a Pasteur pipette to a tissue bath (0.5 ml volume) having a Sylgard-coated bottom, pinned down and superfused with Tyrode solution at $3\ ml\ min^{-1}$ using a peristaltic pump. The bath temperature was 36.0 ± 0.5°C and the Tyrode solution was gassed with 95% O_2 and 5% CO_2 (pH 7.4). The Tyrode solution had the following composition (in mM): NaCl 127, KCl 5.4, NaH_2PO_4 0.43, $MgCl_2$ 1.0, $NaHCO_3$ 23.8, $CaCl_2$ 0.6, glucose 5.55. The Ca^{2+} concentration was lower than usual in order to reduce the contractile force and facilitate long-term cell impalements.

Electrophysiological techniques

The microelectrodes, made of Kwik-fil glass capillaries (WPI) were filled with 3 M KCl and had resistances of 20–40 MΩ. Ag: AgCl half-cells were used. The microelectrodes were connected to a Dagan preamplifier (model 8500). In some experiments, intracellular current pulses were applied through the microelectrode using a bridge circuit (in the preamplifier) to hyperpolarize the cells. When necessary, extracellular field stimulation with pulses of 50 V, 5 ms duration and 0.5–1 Hz, was applied through two platinum plate electrodes connected to a Grass stimulator (S4) with a stimulus-isolation unit. The maximum rate of rise of the action potential (V_{max}) was determined electronically using an OP-AMP circuit with time-constant of 100 μs. Both the action potential and V_{max} were displayed on a Tektronix 5111 storage oscilloscope and photographed with a Grass kymograph camera. The photographic records were enlarged (7x) and the following parameters measured: maximum diastolic potential (MDP), action potential amplitude (APA), overshoot of action potential, duration of action potential from its peak to 50% repolarization (APD_{50}) and V_{max}.

Protocols and drugs

Spontaneously-beating preparations were allowed to equilibrate in the experimental chamber for 30–40 min in order to adapt to the new conditions. After recording stable control action potentials over a 5–10 min period, drugs were added to the Tyrode solution in increasing concentrations (cumulative doses) ranging from 10^{-5} to 10^{-3} M. Steady-state effects were recorded 15–20 min after the beginning of the perfusion with each concentration.

The drugs used were: (−)-adrenaline (Sigma); histamine hydrochloride (Sigma); (−)-isoprenaline hydrochloride (Sigma); lidocaine (Sigma); ouabain octahydrate (Sigma); procaine hydrochloride (Sigma); procainamide hydrochloride (Sigma); propranolol hydrochloride (I.C.I.); quinidine hydrochloride (Sigma); strontium chloride (K & K), and verapamil (Knoll).

Results

Effects of lidocaine, procaine, procainamide and quinidine on pacemaker and action potentials of organ cultured ventricular cells

In 5 experiments lidocaine, 10^{-5} M, diminished slightly the rate of pacemaker discharge and V_{max} (Figure 1b). A concentration of 5×10^{-5} M reduced APA, V_{max}, overshoot, and APD_{50}, with minimal alterations in the MDP (Figures 1c, 2 and 3). Raising the lidocaine concentration to 10^{-4} M invariably blocked the spontaneous discharges, but the preparations could still be driven by electrical field stimulation (Figure 1e). The stimulated electrical activity was completely blocked at 10^{-3} M without noticeable changes in the MDP (not shown). All these effects were completely reversed after washing for 30–40 min.

Superfusion with procaine, 5×10^{-5} M, induced effects qualitatively similar to those found with lidocaine (Figure 1g). However, elevated concentration of procaine (10^{-4} M) induced a phenomenon quite different from that seen with lidocaine in 6 out of 9 experiments. The cells, after brief periods of normal discharge, suddenly depolarized to potentials around −30 to −35 mV, remained there for 3–8 s then came back to the original diastolic potential and oscillated between these two states of membrane potential for long periods of time (5–10 min) (Figure 1h). The

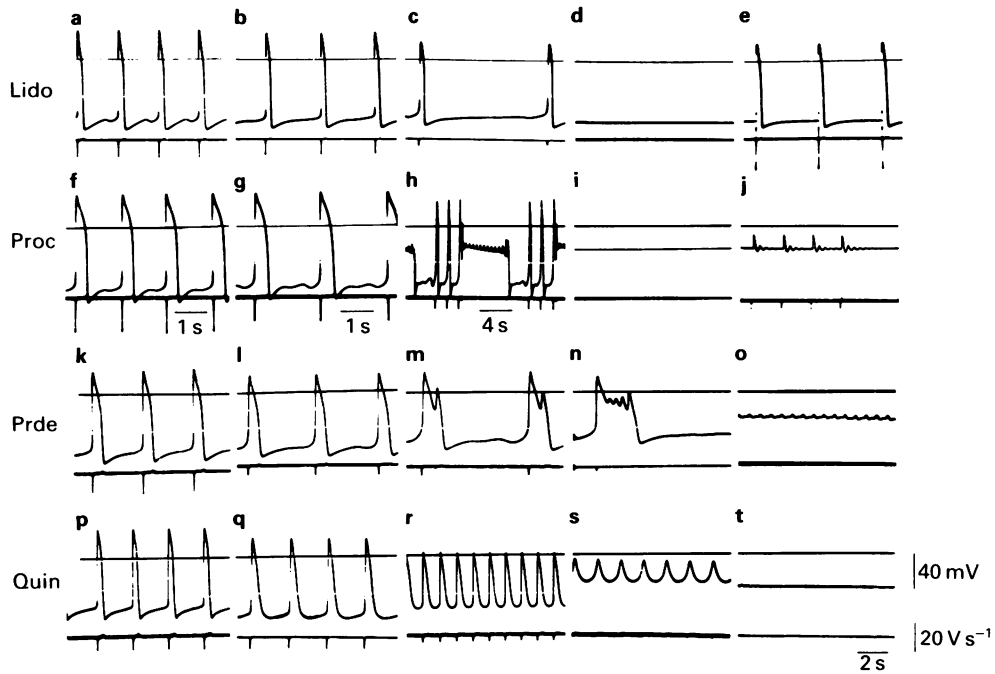

Figure 1 Effects of lidocaine (Lido), procaine (Proc), procainamide (Prde) and quinidine (Quin) on electrical activity of organ cultured embryonic chick ventricular cells (2–3 day-old). The first column (a,f,k,p) shows control spontaneous activity of four different preparations. First row: effects of lidocaine 10^{-5} M (b), 5×10^{-5} M (c) and 10^{-4} M (d). Panels (b) (c) and (d) were separated by 15–20 min intervals. In (c) taken 2 min after (d), the preparation bathed by 10^{-4} M lidocaine was driven by electrical field stimulation. The same cell was impaled in (c), (d) and (e). Second row: procaine 5×10^{-5} M (g), 10^{-4} M (h) and 5×10^{-4} M (i); (j) procaine plus electrical field stimulation; all records are from the same cell. Third row: procainamide 10^{-5} M (l), 5×10^{-5} M (m), 10^{-4} M (n) and 5×10^{-4} M (o), separated by 15–20 min intervals; (m), (n) and (o) are from the same cell. Fourth row: quinidine 10^{-5} M (q), 5×10^{-5} M (r), 10^{-4} M (s) and 5×10^{-4} M (t); (r), (s) and (t) are from the same cell. The traces in each panel from top to bottom represent reference potential, membrane potential, and dV/dt. Time, voltage and dV/dt calibrations at the bottom right apply to all panels with the exception of time calibrations in (f), (g) and (h).

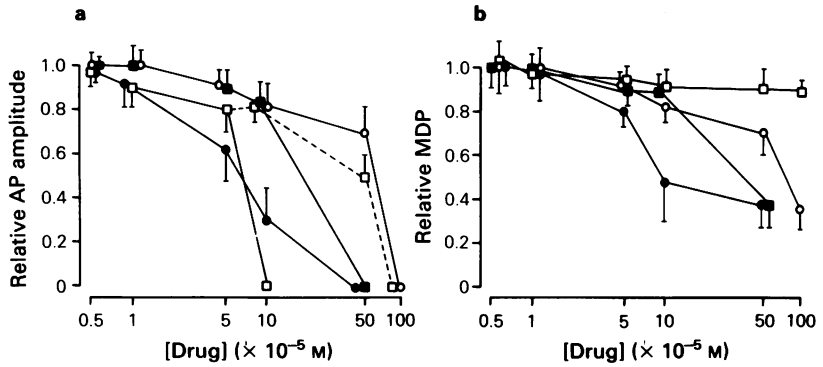

Figure 2 Dose-dependent curves for lidocaine (□; $n = 5$), procaine (■; $n = 6$), procainamide (○; $n = 4$) and quinidine (●; $n = 4$) on the relative maximum diastolic potentials (MDP) (b) and amplitude of spontaneous action potentials (AP) (a) in organ cultured embryonic chick heart cells (2–3 day-old). Each point represents the mean, and vertical lines s.e.means, for n number of hearts. The dashed line represents the effects on AP amplitude in electrically driven preparations.

spontaneous discharges showed reduced APA, overshoot and V_{max} (Figures 1, 2 and 3). Procaine, $5 \times 10^{-}$ M, usually depolarized the cells to membrane potentials around -30 mV, and strong field stimulation induced only weak responses (Figure 1j). Complete recovery occurred upon washing.

A higher concentration of procainamide (5×10^{-5} M) was necessary to induce significant effects on APA, overshoot, and APD_{50}. The rate of depolarization of the ventricular cells, however, was reduced at 10^{-5} M (Figures 1 and 3). Greater concentrations (10^{-4}–10^{-3} M) gradually affected the repolarization, and oscillations at the plateau level were seen in all 4 preparations studied (Figure 1m,n). The cells, finally, could no longer repolarize, their membrane potentials remained around -35 to -30 mV, and complete inexcitability resulted. These effects were also completely reversed after washing.

Quinidine, 10^{-5} to 10^{-4} M, gradually depolarized the cells, reduced APA, overshoot, V_{max} and APD_{50} in four experiments (Figures 1 q–s, 2 and 3). In contrast to the other agents tested, quinidine increased the frequency of spontaneous discharge. Quinidine, 5×10^{-4} M, blocked the action potentials, and the cells remained in a depolarized state (Figure 1t). All these effects were reversed completely after about 40–50 min of superfusion with drug-free Tyrode solution.

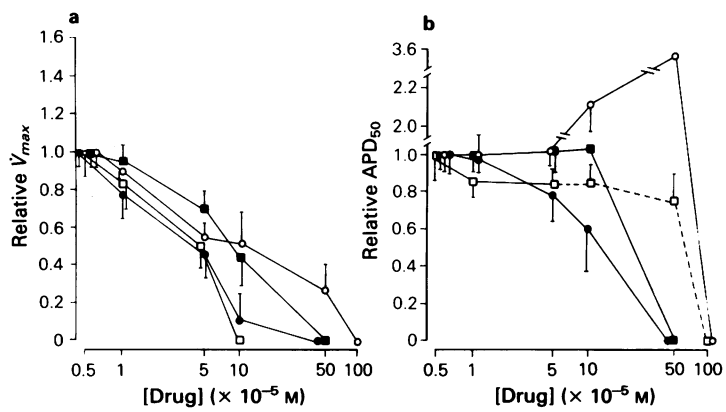

Figure 3 Dose-dependent curves for lidocaine (□; $n = 5$), procaine (■; $n = 6$), procainamide (○; $n = 4$) and quinidine (●; $n = 4$) on (a) the relative maximum rate of rise (V_{max}) and (b) action potential duration at 50% repolarization (APD_{50}) of spontaneous action potentials in organ cultured embryonic chick heart cells. Each point represents the mean, and vertical lines s.e.means, for n number of hearts.

Interaction of the local anaesthetic and antiarrhythmic agents with β-adrenoceptor agonists, histamine and ouabain

Addition of adrenaline (5×10^{-7} M) or isoprenaline (10^{-7} M) to the Tyrode solution increased the frequency of spontaneous discharge, APA, and V_{max} in three experiments. It also appeared, through microscopic observation, that there was an increase in the contractile force of the ventricular cells after catecholamine administration.

Isoprenaline (5×10^{-7} M) was able to restore spontaneous discharge in cells whose activity had been blocked by previous application of local anaesthetic-antiarrhythmic agents. As shown in Figure 4h, 3–5 min after the addition of isoprenaline to the solution containing 10^{-4} M lidocaine, the cells resumed their spontaneous electrical activity. The blocking effect induced by higher concentrations of lidocaine (10^{-3} M) in electrically-driven cells was not, however, reversed by isoprenaline (10^{-6} M) in two experiments.

In cells previously depolarized by procaine (5×10^{-4} M; $n = 4$) (Figure 4a–e), procainamide (10^{-3} M; $n = 3$) or quinidine (5×10^{-4} M; $n = 3$), both β-adrenoceptor agonists, isoprenaline (5×10^{-7} M; $n = 8$) and adrenaline (10^{-6} M; $n = 2$), were invariably able to restore the membrane potentials to the resting levels and to re-establish spontaneous discharges. It was noted (two experiments) that the previous application of isoprenaline (5×10^{-7} M) prevented the depolarization and the electrical inexcitability induced by 5×10^{-4} M procaine.

Propranolol (5×10^{-7} M), applied concomitantly with procaine (5×10^{-4} M), did not affect the phenomena induced by this local anaesthetic, but prevented their reversal by isoprenaline (three experiments).

Histamine (10^{-5} M) was not able to reverse the effects induced by previous application of 5×10^{-4} M procaine (three experiments). A similar lack of restorative effect upon the procaine-induced depolarization was shown by $SrCl_2$ (10 mM; $n = 2$).

We analysed whether the depolarization induced by procaine, and its reversal by isoprenaline, was the result of actions involving the Na/K-pump (four experiments). Superfusion with ouabain (10^{-6} M) for 15 min induced only a slight depolarization (2–4 mV). Addition of procaine (5×10^{-4} M) to the ouabain-containing solution induced effects similar to those observed when procaine was perfused alone. Isoprenaline (5×10^{-7} M) was still able to reverse the effects of procaine obtained in the presence of ouabain in all four preparations.

Comparison with verapamil

Verapamil (10^{-5} M) was superfused in three experiments until its depolarizing and blocking effects on the cells were established. After 15 min, the cells had resting potentials of about − 40 mV, absence of

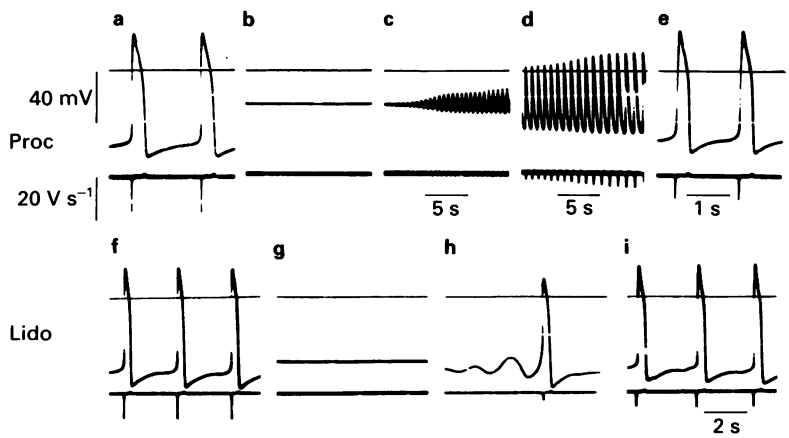

Figure 4 Effects of isoprenaline (5×10^{-7} M) on organ cultured embryonic chick ventricular cells previously treated by procaine (5×10^{-4} M) or lidocaine (10^{-4} M). (a) and (f) Show control spontaneous action potentials in two different preparations. Upper traces: the effect of perfusion with procaine (Proc) for (b) 10 min, (c) 4 min (d) 5 min and (e) 10 min after the addition of isoprenaline to the solution containing procaine; (b), (c) and (d) are from the same cell. Lower traces: the effect of perfusion with lidocaine (Lido) for (g) 12 min, (h) 3 min and (i) 8 min after addition of isoprenaline to the solution containing lidocaine; (g) and (h) are from the same cell. In each panel, the traces represent, from top to bottom, reference potential, membrane potential, and dV/dt. Voltage and V_{max} calibrations apply to all panels. Time calibration in (e) applies also to (a) and (b), and that in (i) applies to (f–i).

pacemaker potentials, and were completely inexcitable by strong electrical stimulation. In clear contrast with the results observed in preparations treated with local anaesthetics and antiarrhythmics, isoprenaline, even at 10^{-6} M, did not reverse the verapamil effects.

The effect of hyperpolarization on the blocking actions of procaine, procainamide or quinidine

As shown in Figure 5, hyperpolarization to pre-drug levels of membrane potential for 10–15 s did not reverse the blocking effects induced by procaine (5×10^{-4} M; $n = 3$). The absence of an effect with the hyperpolarizing current was also found with procainamide (10^{-3} M; $n = 2$) and quinidine (5×10^{-4} M; $n = 2$).

Effect of procaine on the membrane input resistance

Hyperpolarizing current pulses of 10^{-8} A and 50–100 ms duration were injected into impaled cells superfused with procaine (5×10^{-4} M) in order to measure the input resistance (R_{in}) before and during the drug-induced depolarization. The preparations were initially perfused with procaine, and subsequently a stable impalement was obtained. Values of R_{in} measured at the MDP (around − 55 to − 60 mV) were compared with those at the full depolarized level (around − 30 mV) (Figure 6). It was possible to obtain stable impalements and unaltered bridge balance throughout the experiment in four hearts. The input resistance increased from a mean value of 1.83 ± 0.29 MΩ (± s.e.mean) to 2.6 ± 0.53 MΩ in three hearts, and apparently did not change in another one (1.6 MΩ).

Discussion

Although the final effect of the highest concentrations of local anaesthetic and antiarrhythmic agents on 2–3 day-old cultured chick ventricle cells was a total suppression of pacemaker potentials and action potentials (APs), a distinct pattern of action could be observed between these agents once their concentrations were increased sufficiently. Lidocaine (10^{-5} M) reduced the spontaneous rate of discharge and V_{max} of the cells. Greater concentrations also decreased APA, overshoot, and APD_{50} in a dose-dependent manner, with only a minor reduction in the MDP. After arrest of the preparations by 10^{-4} M lidocaine, external stimuli could still induce APs of nearly normal configuration. This indicates that the pacemaker mechanisms in young embryonic heart cells are more sensitive to the depressant effects of lidocaine than the mechanisms responsible for the spike. In canine (Bigger & Mandel, 1970) and sheep (Weld & Bigger, 1976), spontaneously beating cardiac Purkinje fibres, lidocaine (10^{-6} M–10^{-5} M) also suppressed automaticity, but normal APs could still be induced by electrical stimulation. In contrast with the effects found for lidocaine, automaticity of the 3 day-old chick heart cell is not greatly impaired by Ca antagonistic drugs like verapamil, D-600 and nifedipine that strongly depress their AP characteristics (Kojima & Sperelakis, 1983). These facts suggest that different mechanisms may be responsible for the diastolic depolarization and for the upstroke of the AP in young embryonic heart cells.

There is some controversy regarding the effect of lidocaine on slow responses. Concentrations around 10^{-5} M were found to depress Ca^{2+}-mediated APs in

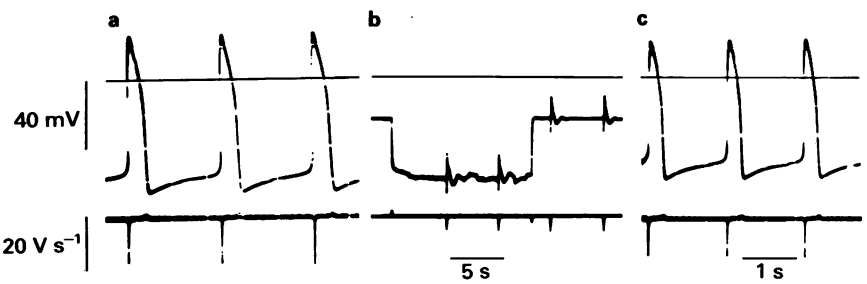

Figure 5 Effect of hyperpolarization on procaine-depolarized organ cultured embryonic chick ventricular cells (2–3 day-old). (a) Control; (b) 10 min after beginning perfusion with procaine (5×10^{-4} M). During and after the hyperpolarizing current pulse, the preparation was stimulated twice in each case, by strong electrical field stimulation; (c) 30 min after washing with procaine-free Tyrode solution.

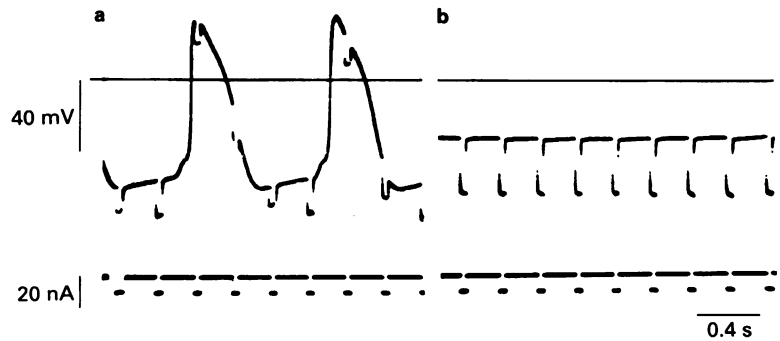

Figure 6 Input resistance obtained by injection of hyperpolarizing constant-current pulses into a cell in an organ cultured embryonic chick heart (2-3 day-old) before (a) and during (b) arrest of the spontaneous action potentials and depolarization by procaine (5×10^{-4} M). (a) Recorded 4 min after the beginning of superfusion of the preparation with procaine; (b) the same cell 12 min later. Note the increase in magnitude of the voltage deflections during the pacemaker potentials in (a), and the larger deflections in (b) compared to (a).

perfused intact chick embryo hearts or reaggregate cell cultures (Josephson & Sperelakis, 1976). Equivalent concentrations impair slow AP conduction through depolarized canine Purkinje fibres (Lamanna *et al.*, 1982). Higher concentrations of lidocaine (2×10^{-4} M) depress the electrical activity that depends on slow inward currents, as in rabbit sinus node cells (Satoh & Hashimoto, 1984) and in canine nodal cells (Satoh, 1981). On the other hand, it has been shown that lidocaine suppresses depressed fast responses, but has little or no effect on slow APs on canine Purkinje fibres (Brennan *et al.*, 1978) and on guinea-pig atrial muscle (Hashimoto *et al.*, 1979).

Our results show that 10^{-5}–10^{-4} M lidocaine depresses or blocks slow APs of young embryonic heart cells. These action potentials are thought to be due to an influx of Na^+ ions through slow channels (Sperelakis & Shigenobu, 1972; Shigenobu *et al.*, 1974) that are blocked by verapamil and D-600 but resistant to TTX and 1 mM Mn^{2+} (Sperelakis & Lehmkuhl, 1965; McLean *et al.*, 1974; Kojima & Sperelakis, 1983).

Procainamide and quinidine (10^{-5} M) have been shown to depress spontaneous diastolic depolarization of canine (Hoffmann, 1957; Rosen *et al.*, 1972; 1973) and sheep Purkinje fibres (Weidmann, 1955; Arnsdorf & Bigger, 1976). In an extensive study in rabbit sino-atrial nodal cells, Senami & Irisawa (1981) showed that procainamide (10^{-4} M) induced a reduction of the MDP (to about − 30 mV), V_{max}, APA, and an increase in the AP duration. Under voltage-clamp conditions, there was a decrease in both transient inward and outward currents without a change in their kinetics. It was suggested that the reduction of the resting potential was due to a decrease in P_K (Senami & Irisawa, 1981). In cat papillary muscle fibres, quinidine (10^{-4} M) reduced the resting membrane potential, V_{max} and prolonged the late repolarization phase. Voltage-clamp data showed that both slow inward and outward currents were reduced by quinidine (Nawrath, 1981). Similar findings were obtained in frog atrial fibres (Ducouret, 1976).

The present results show that procaine, procainamide and quinidine, in a dose-dependent manner, depolarize cultured embryonic heart cells with a concomitant reduction in APA, V_{max} and overshoot. In addition, procainamide and procaine increased the AP duration, inducing oscillations at the plateau level. These effects could be explained if we assume a drug-induced decrease in both slow inward and outward currents in young embryonic cells. These agents probably also caused a decrease in resting P_K which would be responsible for the pronounced fall in the resting potential. This assumption is strengthened by the increased R_{in} during the procaine-induced depolarization. On the other hand, the results obtained with the perfusion of ouabain alone or in combination with procaine, suggest that a possible inhibition of the Na/K-pump is not a primary mechanism in the depolarization. The results obtained by long hyperpolarizing pulses in depolarized procaine-treated cells, show that a direct blocking effect upon slow Na^+ channels is probably additional to an indirect effect, due to a depolarization-induced inactivation of I_{si}, in producing the dose-dependent fall in APA and V_{max}.

In our experiments, quinidine increased the frequency of spontaneous discharges concomitant with the depolarizing effect. It has been found previously (Sperelakis & Lehmkuhl, 1968) that cocaine and tetracaine (0.3–0.7 mM) depolarize and increase the firing in monolayer cultures of 7–10 day-old embryonic chick heart cells. In the case of these

three agents, the expected acceleratory response of a pacemaker cell to depolarization seemed to surpass an eventual depressor effect by these drugs on the slow diastolic depolarization.

Isoprenaline and adrenaline have been shown to reverse the effects of local anaesthetics and antiarrhythmic agents on embryonic cells. This antagonism was probably carried out through β-adrenoceptor stimulation, since it was prevented by propranolol. It is known that isoprenaline activates adenylate cyclase and increases adenosine 3′:5′-cyclic monophosphate (cyclic AMP) levels in young cultured and non-cultured chick embryonic hearts (Polson *et al.*, 1977; Renaud *et al.*, 1978; Alexander *et al.*, 1982). Although direct evidence in our experiments is lacking, it could be speculated that activation of adenylate cyclase does not seem to be involved in the isoprenaline-induced repolarization since histamine, which is also known to increase cyclic AMP levels in other heart tissues (Watanabe & Besch, 1974) did not reproduce this effect. Another agent, strontium, which has been found to hyperpolarize and restore the electrical activity of monolayer cultures of heart cells depolarized by cocaine and tetracaine (Sperelakis & Lehmkuhl, 1968) had no similar action on cultured 2–3 day-old embryo heart cells. Since hyperpolarization alone was not sufficient to antagonize the blocking effects of procaine, isoprenaline and adrenaline probably operate through a combination of actions having in common β-adrenoreceptor stimulation. The reversal of the blocking effects of lidocaine could be explained by the well-known actions of the β-adrenoceptor agonists on the rate of slow diastolic depolarization and the magnitude of I_{si}. An additional catecholamine-induced increase in P_K in preparations depolarized by procaine, procainamide and quinidine would eventually bring their resting potential back to normal levels and cause a return to normal firing.

Isoprenaline or noradrenaline, at nanomolar concentrations, have been shown to induce hyperpolarization in cardiac Purkinje fibres, not by increasing Na/K-pump current, but by increasing resting membrane P_K (Gadsby, 1983). This is in keeping with the lack of a preventive effect of ouabain on the isoprenaline-induced reversal of procaine-treated cells found in the present investigation. However, it does not preclude the possibility that pump stimulation could be an additional mechanism in the catecholamine-induced repolarization. In cat papillary muscle, isoprenaline (10^{-6} M) also reverses the blockade of I_{si} induced by quinidine (Nawrath, 1981). The blocking action of verapamil on the electrical activity of ventricular cells is certainly different from the effects of the local anaesthetics and antiarrhythmic agents since it was not reversed by β-adrenoceptor agonists.

Despite the obvious similarities, each of these drugs studied showed peculiarities of action on the electrical activity of cultured cells. This has already been pointed out by other investigators studying these agents (Arnsdorf & Bigger, 1975; 1976; Carmeliet & Saikawa, 1982; Colatsky, 1982). It should be emphasized, however, that the preparation used here showed one to two orders of magnitude less sensitivity to the antiarrhythmic agents than the mammalian preparations frequently used in other investigations.

In conclusion, local anaesthetics and antiarrhythmic agents depress, in a dose-dependent manner; pacemaker and action potentials of organ cultured 2–3 day-old embryonic chick heart cells. Judging from their effects on the slow diastolic depolarization and on V_{max} of the slow Na^+ channel-dependent action potentials, it seems reasonable to assume that these channels have a sensitivity to these agents similar to that shown by the Ca^{2+}-dependent slow APs of reaggregate cell cultures and intact embryonic (16–20 day-old) chick hearts.

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