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A comparison between the negative inotropic action of various antiarrhythmic drugs and their influence on calcium movements in heart muscle *

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The influence of quinidine, lidocaine, (+)- and (±)-propranolol, L-(+)- and D-(−)-INPEA and (±)-alprenolol on contractile force and transmembraneous calcium exchange was studied in beating guinea-pig atria (3Hz) and in resting left auricles. Quinidine and lidocaine (5×10^{-5}M) reduced the contractile force by approximately 34 and 19%, respectively. Both drugs diminished the Ca^{2+} flux during excitation without affecting the exchangeable fraction at equilibrium or the total Ca^{2+} content. In resting auricles both drugs did not influence the passive Ca permeability whatsoever. (+)- or (±)-Propranolol and alprenolol (5×10^{-5}M) depressed the contractile force by approximately 60 and 33% of the initial value, INPEA (both isomers) hardly showed negative inotropic properties. None of these β-adrenoceptor blocking agents significantly affected the calcium fluxes or the total Ca^{2+} content, either in beating atria or in resting auricles. The negative inotropic action of quinidine and lidocaine might be explained at least in part by the reduced rate of transmembraneous calcium exchange. For the β-adrenoceptor blocking compounds such a mechanism cannot be the cause of their depression of cardiac contractile force. It is suggested that the expression "quinidine like" effect is not adequate for the general description of the negative inotropic side effect of most β-adrenoceptor blocking and related drugs.

Most antiarrhythmic drugs possess negative chronotropic and inotropic properties. Whereas the decrease in frequency may be explained satisfactorily by changes in membrane permeability of pacemaker cells and corresponding deformation of the action potential (for review see Vaughan Williams, 1964), the negative inotropic action is not well understood. Changes in calcium metabolism may be involved in the development of the negative inotropic action of quinidine (Rahn & Reuter, 1966). However, few quantitative studies on calcium exchangeability in the presence of quinidine and related drugs have been made in intact, mechanically active heart muscle preparations. To demonstrate a reduction in calcium exchange in isolated sarcoplasmic reticulum preparations, high concentrations of quinidine or propranolol are required and to relate the importance of such findings to phenomena *in vivo* is difficult (for literature see "Discussion"). For this reason we have examined the influence of quinidine, lidocaine, (+)- and (±)-propranolol and L-(+)- and

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D-(−)-INPEA on the rate of exchange between extracellular $^{45}Ca^{2+}$ and cellular calcium in electrically driven, guinea-pig isolated atria. Negative inotropic effects have also been assessed.

METHODS

The atria of guinea-pigs, 250–350 g of either sex were obtained according to Hoditz & Lüllmann (1964). The organ bath, a 1000 ml beaker, contained 750 ml of normal Muralt-Tyrode solution (1·8 mequiv Ca^{2+}/litre) at 30° gassed with 5% carbon dioxide in oxygen. The atria were stimulated supramaximally by means of a Grass S 4 H stimulation device at 3 Hz with rectangular pulses of 3 ms duration. In separate experiments the effects of the drug on non-stimulated left auricle preparations were also studied.

(a) *Stimulated atrial preparations.* After equilibration in Muralt-Tyrode solution for 30 min, stimulated preparations were preincubated for 20 min with an appropriate concentration of drug to allow the development of the negative inotropic action. The preparations were then transferred to a solution containing both the drug and approximately 50 μCi ^{45}Ca/litre and further incubated for fixed periods ranging from 15–180 min.

Control atrial preparations were similarly incubated for comparison, but without the addition of drug. Not less than eight separate experiments formed the basis of each result presented.

(b) *Unstimulated left auricle preparations.* To enable a comparison with stimulated preparations, left auricle tissues were similarly treated as described above with the exception that the preparation was not electrically stimulated during the incubation period. Not less than eight separate experiments formed the result summaries presented in this paper. Control left auricle preparations were similarly incubated for comparison, but without the addition of drug.

The tissues were blotted dry and gently pressed between "Blauband" filter paper under standardized conditions. Their [$^{45}Ca^{2+}$] content and total calcium concentration were subsequently determined according to Lahrtz, Lüllmann & van Zwieten, 1967, and Haacke, Lüllmann & van Zwieten (1970). The [$^{45}Ca^{2+}$] content of the isolated tissue was converted into the amount of Ca^{2+} that had exchanged with the total calcium content and, after correction for the extracellular space, expressed as nequiv Ca^{2+}/100 mg cellular material wet weight. The extracellular space was determined by means of [^{14}C]sucrose (Lüllmann & van Zwieten, 1967) and found to be 0·35 ml/g tissue both in beating atria (3 Hz) and in resting left auricles. Quinidine or lidocaine (5×10^{-5}M) did not reduce this value of the extracellular space in spite of their negative inotropic actions.

In separate experiments the negative inotropic action of the drugs was determined in atria electrically stimulated at 3 Hz. The mechanical activity was recorded using a strain gauge, connected to a Hellige (type HE 86t) amplifier and corresponding recorder. The negative inotropic action was expressed as percentage of the initial value of the contractile force.

Drugs. Quinidine sulphate (Merck AG, Darmstadt), lidocaine hydrochloride (Astra AB., Södertalje/Sweden), (+)- and (±)-propranolol hydrochloride (Rheinpharma, Heidelberg, L(+)- and D(−) INPEA (Selvi & Co., Milan), (±)-alprenolol hydrochloride (Astra AB., Södertalje/Sweden). $^{45}CaCl_2$ and [^{14}C]saccharose were obtained from the Radiochemical Centre, Amersham (Bucks.).

RESULTS

Negative inotropic action

The negative inotropic action of the drugs was quantitatively determined using the same concentrations for the investigation of the compounds as those used for calcium movement. The results are summarized in Table 1.

Table 1. *Negative inotropic effects of quinidine, lidocaine,* (+)- and (±)-*propranolol,* (±)-*alprenolol and* L (+)- *and* D (−)-*INPEA on electrically driven guinea-pig isolated atria* (*stimulation* 3 *Hz*). The maximum reduction in contractile force in the presence of antiarrhythmic drug is expressed as a percentage of the initial response mean ± s.e. for n different atria.

Drug	Concentration (M)	n	Mean decrease in contractile force (% of original response (± s.e.)
Quinidine	5×10^{-5}	8	34 ± 6
Quinidine	5×10^{-4}	2	100% (all mech. act. stopped)
Lidocaine	5×10^{-5}	4	19 ± 3
(±)-Propranolol	5×10^{-5}	7	64 ± 7
(+)-Propranolol	5×10^{-5}	7	56 ± 6
(±)-Propranolol	5×10^{-4}	2	100% (all mech. act. stopped)
L-(+)-INPEA	5×10^{-5}	4	7 ± 1
D-(−)-INPEA	5×10^{-5}	6	7 ± 1
(±)-Alprenolol	5×10^{-5}	5	3 ± 4

The negative inotropic action of both INPEA isomers was weak compared with that of the other drugs and was not significantly increased by a tenfold rise in dose. None of the drugs reduced the electrically-imposed frequency of beating, i.e. no dropped beats were observed. Both quinidine and (±)-propranolol (5×10^{-4}M) stopped mechanical activity which was not accompanied by any evidence of induced contracture. Compared with the other drugs the negative inotropic action of quinidine developed slowly, requiring about 20 min to attain its maximum effect whereas with lidocaine and INPEA (both isomers) the effect developed in 10 min and for both isomers of propranolol in 12–15 min.

Influence on calcium exchange

Quinidine (5×10^{-5}M) much reduced the rate of the exchange between extracellular ^{45}Ca and cellular calcium in beating atria (frequency 3 Hz). The difference in [^{45}Ca]-content between controls and quinidine-treated atria was statistically significant at periods of 30, 60 or 120 min of incubation ($0{\cdot}01 < P < 0{\cdot}05$, Student's *t*-test). Neither the total calcium content of the atria nor the exchangeable fraction at equilibrium were influenced by the presence of the drug (5×10^{-5}M); thus the upper line in Fig. 1 represents the mean value (± s.e.) for both control and quinidine-treated atria. In resting left auricles neither the rate of calcium exchange nor the total calcium content were influenced by the drug (5×10^{-5} or 5×10^{-4}M).

At 15 and 60 min incubation where in beating atria a large difference was seen, the same values for [^{45}Ca]uptake were found in both control and quinidine-treated atria (*see* Fig. 2). At 5×10^{-4}M, quinidine inhibited mechanical activity in beating atria.

Lidocaine (5×10^{-5}M) also reduced the rate of calcium exchange in beating atria, without affecting the exchangeable fraction and the total calcium content in the same

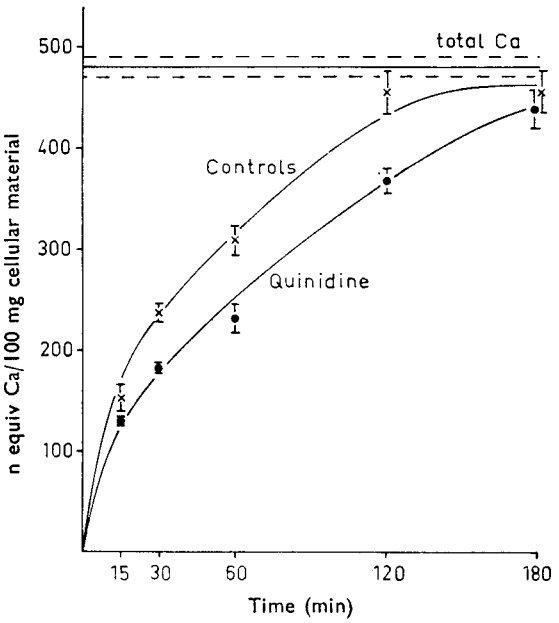

FIG. 1. Influence of quinidine (5×10^{-5}M) on the uptake of extracellular $^{45}Ca^{2+}$ by electrically stimulated guinea-pig atria (3Hz). The amount of calcium that had exchanged was calculated from the $^{45}Ca^{2+}$ content of the organs and subsequently corrected for the extracellular space. Each point on the curves represents the mean value (± s.e.) for at least 8 different atria. The total calcium content of all atria (controls and quinidine) is represented by the straight line parallel to the abscissa (mean ± s.e.).

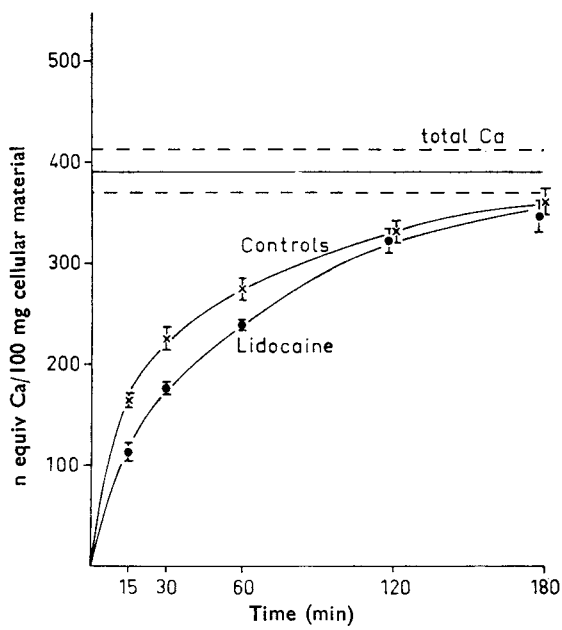

FIG. 2. Influence of lidocaine (5×10^{-5}M) on the uptake of extracellular $^{45}Ca^{2+}$ by electrically stimulated guinea-pig atria (3 Hz). Details as in Fig. 1.

atria (see Fig. 2). At 15, 30 or 60 min incubation the differences were statistically significant ($0{\cdot}01 < P < 0{\cdot}05$). As for quinidine, the calcium metabolism in resting left auricles was not influenced.

(+)- and (±)-Propranolol did not influence the uptake of ^{45}Ca after either 15 or 60 min incubation, in spite of the pronounced negative inotropic action of both compounds. The highest concentration (5×10^{-4}M) which stopped mechanical activity in beating atria did not influence the exchange of calcium in resting left auricles, nor their total Ca^{2+} content. Similarly, both isomers of INPEA at 5×10^{-5}M did not influence the Ca^{2+} exchange at 15 or 60 min incubation, nor the muscle's total content.

(±)-*Alprenolol* (5×10^{-5}M) did not reduce the rate of Ca^{2+}-exchange in beating atria, 15 and 60 min after incubation.

Comparison between the negative inotropic action and the reduction in the rate of Ca^{2+} *exchange*

To allow a comparison between the negative inotropic action and the influence of the drugs on the rate of calcium exchangeability, the *maximum* depression of cellular $^{45}Ca^{2+}$ uptake (obtained from the difference between the two curves, representing $^{45}Ca^{2+}$ uptake under control circumstances and in presence of the drug) was compared with the maximum reduction in contractile force (*cf.* Table 1). The maximum difference was related to the corresponding control value and expressed as percentage of this value (*cf.* Table 1).

The results of this comparison are represented schematically in Fig. 3. From this

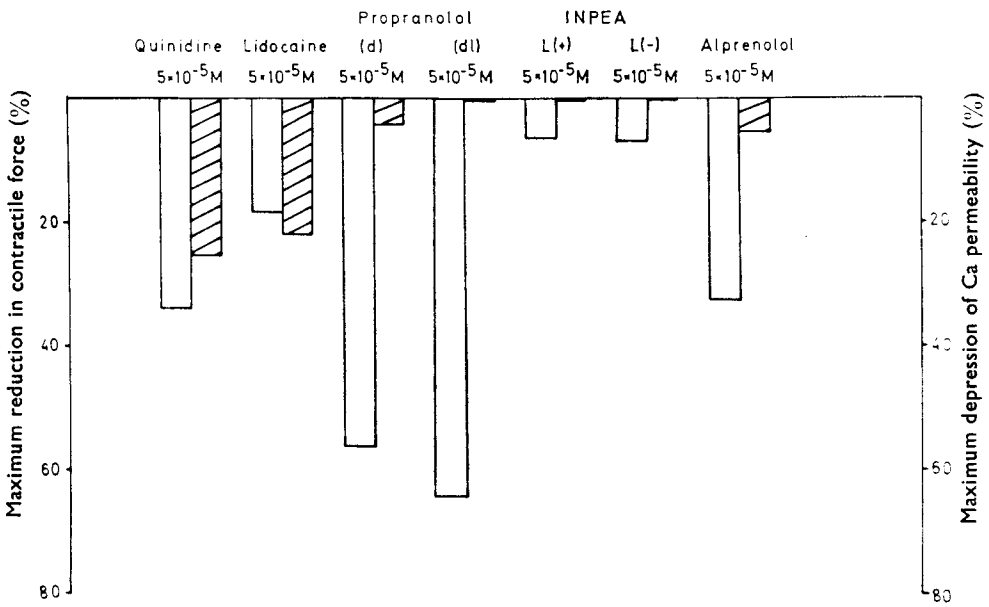

FIG. 3. Comparison of the negative inotropic effects and the depression in calcium permeability caused by quinidine, lidocaine, (+)-and (±)-propranolol, L-(+)- and D-(—)-INPEA and (±)-alprenolol in beating guinea-pig atria. The negative inotropic action was expressed as percentage reduction of the initial contractile force (left ordinate). The maximum difference between the two curves representing Ca^{2+} exchange under control circumstances and in presence of the drug (cf. Figs 1 and 2) was expressed as percentage of the corresponding control value (right ordinate). The lack of correlation between both parameters is obvious for the β-adrenoceptor blocking agents studied.

figure it is obvious that both (+)- and (±)-propranolol and (±)-alprenolol do not influence calcium metabolism in spite of their negative inotropic effects. INPEA (both isomers) hardly influences contractile force and does not reduce Ca^{2+}-exchangeability. Quinidine and lidocaine on the other hand reduce the rate of calcium exchange during excitation and simultaneously reduce contractile force.

DISCUSSION

Upon comparison of the negative inotropic properties of the drugs used the following order of potency holds: (+)- and (±)-propranolol > quinidine > alprenolol > lidocaine > INPEA (Gorino & Shannon, 1969 and, in part, van Zwieten, 1969). Several investigators have described the modest negative inotropic action of lidocaine in various experimental models (Harrison, Sprouse & Morrow, 1963; Jewitt, Kishon & Thomas, 1968; Morales-Aquilera & Vaughan Williams, 1965; Binnion, 1968). In spite of its β-adrenoceptor blocking action, the cardiac depression of both isomers of INPEA is almost negligible (Levy, 1968).

None of the drugs in the concentrations used significantly altered the total calcium content in guinea-pig atrial tissue so that drug-induced changes in $^{45}Ca^{2+}$ uptake must be considered as alterations of Ca^{2+} exchange. Both quinidine and lidocaine reduced the rate of Ca^{2+} exchange without influencing the exchangeable fraction at equilibrium after 3 h incubation (*cf.* Rahn & Reuter, 1966). This finding suggests that both drugs diminish the membrane permeability for Ca^{2+} during excitation. The diminished rate of exchange was only observed during excitation, since even the very high concentrations of quinidine that stopped mechanical activity in beating atria did not influence the passive Ca^{2+} permeability in resting left auricles. This finding shows a similarity to the well-known observation that quinidine inhibits Na^+ and K^+ fluxes during excitation only (Goodford & Vaughan Williams, 1962, review by Vaughan Williams, 1964). Possibly, during excitation quinidine and lidocaine cause a general reduction in membrane permeability for Na^+, K^+ and Ca^{2+} ions that would explain the antiarrhythmic, negative inotropic and local anaesthetic properties of both drugs. Hexobarbitone impairs Ca^{2+} permeability similarly (Haacke & van Zwieten, 1971), but much less so for Na^+ and K^+ ions (*cf.* van Zwieten, 1969). An inhibition in isolated sarcoplasmic reticulum particles from cardiac muscle of $^{45}Ca^{2+}$ exchange by quinidine (Fuchs, Gertz & Briggs, 1968; Shinebourne, White & Hamer, 1969) and by lidocaine (Johnson & Inesi, 1969) has been described. These findings, especially as they were brought about by high drug concentrations, are difficult to relate to the negative inotropic effects in intact, contracting heart muscle.

None of the β-adrenoceptor blocking agents influenced the Ca^{2+} influx either during excitation or at rest. Scales & Macintosh (1968) and also Shinebourne & others (1969) observed a reduced $^{45}Ca^{2+}$ uptake in isolated sarcoplasmic reticulum preparations treated with propranolol or INPEA, but at concentrations (up to 10^{-3}M) which are not comparable to those producing an action in intact heart muscle. Moreover, an inhibition of $^{45}Ca^{2+}$ by intracellular structures suggests that more Ca^{2+} would be available for excitation contraction coupling, so that Shinebourne & others (1969) had to postulate an increased cellular efflux of Ca^{2+} to account for the negative inotropic action of propranol. We did not find an increased Ca^{2+} efflux as both total Ca and $^{45}Ca^{2+}$ uptake-exchange were not altered. Changes in Ca^{2+} permeability of the membrane cannot thus satisfactorily explain the negative inotropic properties of the β-adrenoceptor blocking agents we studied. However, in a

model almost identical with ours, Rahn & Reuter (1966) observed a reduced rate of $^{45}Ca^{2+}$ exchange in the presence of 1-(3-methylphenoxy)-2-hydroxy-3-isopropylamino-propane (Kö 592), a β-adrenoceptor blocking agent with strong "quinidine-like" properties. The blockade of cardiac β-adrenoceptors cannot adequately explain the negative inotropic effect either, since D-(−)-INPEA hardly reduced the contractile force although it is a potent β-adrenoceptor blocking agent (Almirante & Murmann, 1966). Moreover, both (±)- and (+)-propranolol provoked virtually the same negative inotropic effect, although the β-adrenoceptor blocking activity of the racemate is much stronger than that of the (+)-form (Barrett & Cullum, 1968).

There was no correlation of the influence of the drugs examined on Ca^{2+} permeability during excitation and their local anaesthetic properties. Moreover, the depression of Ca^{2+}-permeability also seems to be independent on the antiarrhythmic activity, since for some of the drugs we used, Raper & Jowett (1967) found the sequence for anti-arrhythmic potency to be: lidocaine > (±)-propranolol ~ (+)-propranolol > quinidine—a sequence not corresponding to that found for the effects on Ca^{2+}-exchange.

The negative inotropic activity of quinidine and lidocaine may be explained, in part, by a reduction in the rate of transmembraneous Ca^{2+} exchange during excitation, but this does not explain the action of β-adrenoceptor blocking agents. For this reason, the expression "quinidine-like" effect cannot be used generally to characterize the negative inotropic side effects of β-adrenoceptor blocking agents and related drugs (*cf.* Tritthart, Fleckenstein & others, 1969).

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