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Decrease in ionic permeability of the cell membrane in guinea-pig atrial tissue by treatment with antifibrillatory agents and hexobarbitone, determined by means of ^{86}Rb

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1. The influence of antifibrillatory agents on the release of previously accumulated $^{86}Rb^+$ ions was studied in guinea-pig isolated left auricles, incubated in a K^+-free Tyrode solution that contained an equivalent concentration (2.7 mM) of Rb^+. The auricles were stimulated electrically at a frequency of 100/min and showed entirely " normal " mechanical behaviour in the incubation medium.

2. Quinidine, tetracaine, hexobarbitone-Na and propranolol caused a dose-dependent negative inotropic effect. The frequency of beating (100/min) was usually not affected by these drugs.

3. The reduction in contractile force was accompanied by a dose-dependent inhibition of ^{86}Rb release. The concentration-response curves for both the mechanical effect and the influence on ^{86}Rb release almost coincided for quinidine, although these curves diverged for the other drugs studied.

4. It is suggested that all the four different antifibrillatory drugs cause a general reduction of the membrane permeability to inorganic ions. The mechanism of this membrane stabilization remains unknown.

The antifibrillatory action of drugs such as quinidine is thought to result chiefly from the increase of the refractory period (for review see Hoffmann & Matsuda, 1960). The general depression of myocardial activity is believed to be caused by a diminished permeability of the cell membrane to inorganic ions. For quinidine, this decrease in ionic permeability of the membrane has been demonstrated by means of isotope experiments with ^{42}K (Holland, 1957 ; Holland & Klein, 1958 ; Holland, Klein & Briggs, 1959 ; Klein, Holland & Tinsley, 1960). Electrophysiological experiments have shown that quinine inhibits sodium influx during excitation (Lüllmann, 1959). Although most antifibrillatory agents are believed to act by the same mechanism, the influence of such drugs on ion fluxes has usually not been studied quantitatively, probably because the short-lived isotopes ^{42}K and ^{24}Na, necessary for such studies, are difficult to handle. Burgen & Spero (1958), however, have recently shown that ^{86}Rb, an isotope that is much easier to handle than ^{42}K, can be conveniently used as a tracer for the movements of K^+ ions in smooth muscle. It has also been demonstrated that ^{86}Rb can be used to study

the effect of drugs on the membrane permeability to inorganic ions in guinea-pig atrial tissue (van Zwieten, 1968). In order to obtain a better picture of the actions of antifibrillatory agents and of hexobarbitone sodium on membrane permeability, the influence of such agents on the rate of release of previously accumulated $^{86}Rb^+$ ions by guinea-pig isolated auricles was determined quantitatively. The following drugs were investigated: quinidine, tetracaine, hexobarbitone sodium, and propranolol (a β-adrenolytic agent with antifibrillatory properties). The results suggest that all of the various agents investigated give rise to a similar decrease in ionic permeability of the cell membrane.

Methods

Guinea-pig left auricles, dissected according to the procedure described by Hoditz & Lüllmann (1964) were suspended in a potassium-free Tyrode solution that contained an equivalent concentration of Rb^+ ions (2.7 m-moles/l.). The organs were obtained from guinea-pigs of either sex (weight 250–350 g). In this medium the response of the atria to electrical stimulation and to various cardioactive drugs could not be distinguished from that observed in auricles or atria, suspended in " normal," K-containing Muralt-Tyrode solution (van Zwieten, 1968). The Muralt-Tyrode solution was of the following composition: NaCl 137 mM ; KCl 2.7 mM ; $CaCl_2$ 1.0 mM ; $MgCl_2$ 1.0 mM ; $NaHCO_3$ 11.9 mM ; NaH_2PO_4 0.21 mM ; glucose 5.5 mM. In order to load the auricles with ^{86}Rb, part of the Rb in the medium was replaced by ^{86}Rb, until a specific activity of approximately 5 μc/ml. was reached. The volume of the organ bath was 50 ml., and the temperature was maintained at 30° C. The solution was bubbled with 95% oxygen and 5% carbon dioxide. After loading with ^{86}Rb for about 2 hr the auricles were transferred to an organ bath of 1.8 ml. capacity, which was perfused continuously with non-radioactive Rb-Tyrode solution at a rate of 1.5 ml./min. The solution was drawn through the system by means of an influsion pump. The effluent solution, containing ^{86}Rb released from the muscle, passed through a plastic tube (1.8 mm internal diameter), 60 cm of which was coiled within a counting vial mounted in a liquid scintillation counter (ECKO N 664 C). The vial was filled with 25 ml. of phosphor solution (anhydrous toluene containing PPO 4 g/l. and POPOP 0.1 g/l.). The $^{86}Rb^+$ ions, initially accumulated by the auricles were thus exchanged against non-radioactive Rb^+ ions from the perfusion Tyrode solution. The total concentration of Rb^+ in the tissue remained constant throughout the experiment. The $^{86}Rb^+$ ions released by the auricle were constantly removed from the organ bath and led through the tube wound within the counting vial. The counting efficiency was about 75%. The count rate was recorded continuously by means of a Rikadenki recording device, which gave a linear relationship between the ^{86}Rb concentration in the perfusate and the excursion of the recording pen. Consequently, the curve obtained represented the release of $^{86}Rb^+$ ions from the auricle as a function of time. The isolated auricles were stimulated electrically by means of silver electrodes at a frequency of 100/min. The duration of the rectangular impulses was 3 msec, the amplitude 4–8 V. Throughout the experiment oxygenation was carried out by means of 95% oxygen + 5% carbon dioxide, the temperature of the organ bath being kept at 30° C. The auricle in the organ bath was attached to a strain gauge in order to monitor its mechanical activity. The contractions were recorded continuously on a Helcoscriptor device (type HE 86-t).

In order to study the effect of drugs upon the rate of ^{86}Rb release, the perfusion of the organ bath was transiently carried out with a Rb-containing Tyrode solution, to which the drug in question had been added in the appropriate concentration. All drugs used in the present study caused a rapid decline of the $^{86}Rb^+$ concentration in the perfusate. It has been shown previously that a rapidly occurring alteration of the $^{86}Rb^+$ concentration in the perfusate is not accompanied by a significant change in the specific activity of the ^{86}Rb in the auricles. Accordingly, changes in the ^{86}Rb concentration of the perfusate as a result of drug treatment, provided they occur rapidly, may be related directly to changes in Rb efflux from the auricles. In other words, the drug-induced decrease in ^{86}Rb content of the perfusate read on the recorder scale divided by the normal content of ^{86}Rb (as derived from the control curve by extrapolation) represents the relative reduction in $^{86}Rb^+$ efflux as a result of drug treatment (see Fig. 1). Quantitative evaluation of the drug effects was thus carried out by relating the decrease in $^{86}Rb^+$ concentration to the basic concentration in absence of the drug. The drug effects are always expressed as percentage decrease of the basic efflux. It has been shown previously that the Rb efflux of electrically driven atria (frequency 180/min) amounts to 3.2 p-moles Rb^+ cm^{-2} sec^{-1}, that of resting left atria 1.3 p-moles Rb^+ cm^{-2} sec^{-1}. The efflux is directly related to the number of contractions, although independent of the contractile force (van Zwieten, 1968). $^{86}RbCl$ (chromatographically pure) was obtained from the Radiochemical Centre, Amersham. The following drugs were used: quinidine sulphate (Hoechst AG.); hexobarbitone sodium (Bayer AG.); propranolol hydrochloride (I.C.I. Ltd.); tetracaine hydrochloride (Hoechst AG.).

Results

Quinidine

Quinidine caused a dose-dependent reduction of the contractile force that was accompanied by an abrupt decrease of the ^{86}Rb content of the perfusate.

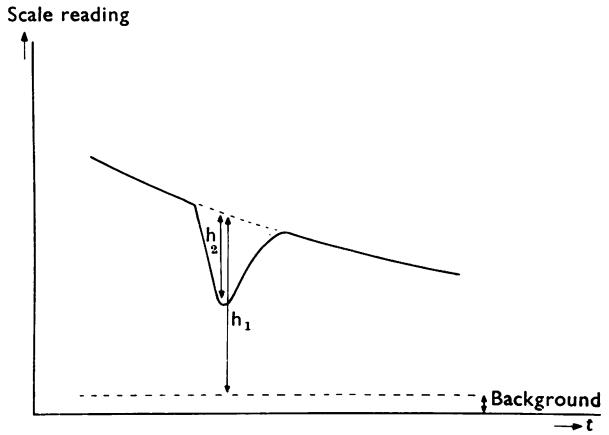

FIG. 1. Schematic representation of the quantitative evaluation of a decrease in $^{86}Rb^+$ efflux, provoked by drugs. The reading on the scale of the recorder indicates the ^{86}Rb content of the perfusate. The dotted line at the bottom indicates the radioactive background. The ratio h_2/h_1 yields the relative decrease of the Rb efflux.

The effect of intermittent exposure of an isolated auricle to quinidine on the concentration of $^{86}Rb^+$ ions in the perfusate is shown in Fig. 2. The effect of quinidine was fully reversible. The dose-effect curve was rather steep (Fig. 3). Whereas a concentration of 10^{-6}M quinidine did not show any negative inotropic

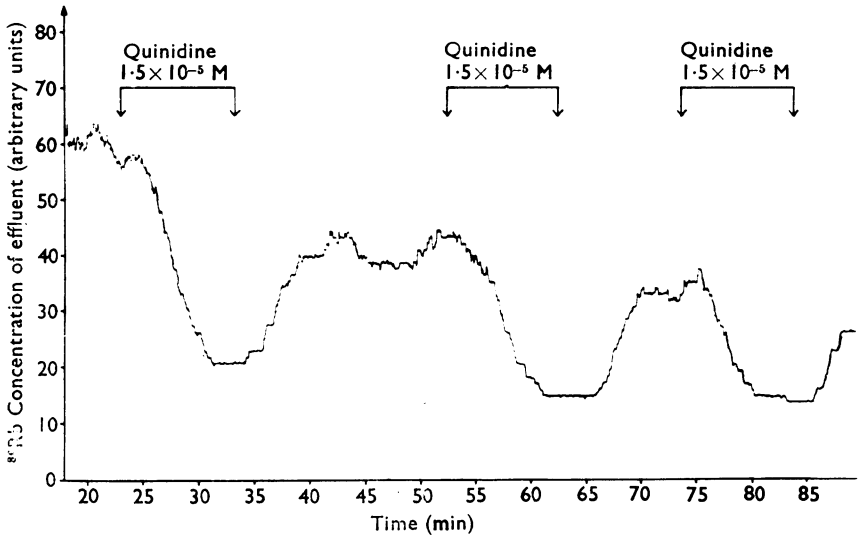

FIG. 2. Inhibition of the $^{86}Rb^+$ release on exposure to quinidine. Isolated left auricles, frequency of beating 100/min. At the times indicated, the organ bath was perfused with a Rb-Tyrode solution containing quinidine (1.5×10^{-5}M).

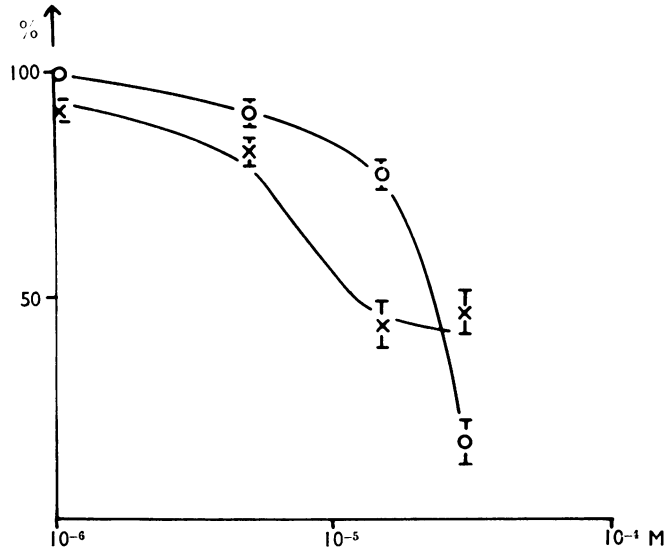

FIG. 3. Reduction of the $^{86}Rb^+$ release (×——×) and the contraction amplitude (○——○) of isolated left auricles by quinidine. Quinidine was added to the perfusion Tyrode solution for periods of 10 min. The $^{86}Rb^+$ efflux and the contraction amplitude were calculated as percentages of the initial values and plotted on the ordinate. Each point on the curves represents the mean effect (±S.E.M.) of eight to ten different perfusions with quinidine in different auricles.

action, a concentration of 3×10^{-5}M reduced the contraction amplitude by about 80%. The relationship between the effects of quinidine in reducing ^{86}Rb efflux and in reducing contractile force is shown in Fig. 3. A rough parallelism between the curves is apparent. Even the highest concentration of quinidine studied (3×10^{-5}M) did not reduce the frequency of beating although it caused a very pronounced negative inotropic effect.

Tetracaine

Tetracaine, a potent local anaesthetic agent, reduced both the contractile force and the rate of ^{86}Rb release of electrically stimulated left auricles. As shown in Fig. 4, both effects were dose-dependent, the dose range being rather narrow. After an initial coincidence the dose response curves of both effects diverged and became significantly different at higher concentrations. As already observed for quinidine,

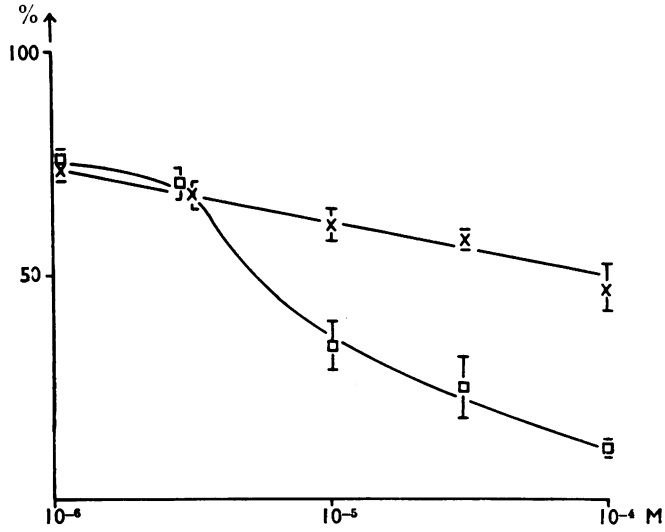

FIG. 4. Inhibition of the ^{86}Rb+ release (×——×) and the contractile force (□——□) on exposure to tetracaine. Details as described in the legend to Fig. 3.

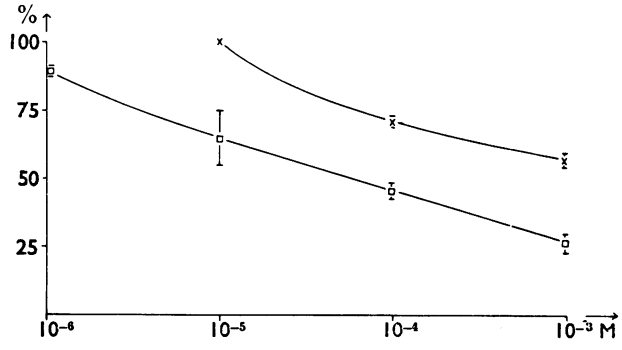

FIG. 5. Inhibition of the ^{86}Rb+ release (×——×) and the contractile force (□——□) on exposure to hexobarbitone sodium. Details as described in the legend to Fig. 3.

even the highest concentration of tetracaine did not diminish the frequency of electrically-induced beating.

Hexobarbitone sodium

Concentrations of hexobarbitone-Na in the range 10^{-5}–10^{-3}M caused a moderate reduction in the contraction amplitude, which was dose-dependent. The inhibition of ^{86}Rb release gave a dose-effect curve that ran almost parallel to the curve obtained for the negative inotropic effect (see Fig. 5). In comparison with quinidine and tetracaine, the barbiturate showed only moderate impairment of both the contractile force and the release of $^{86}Rb^{+}$ ions.

Propranolol

Treatment of the isolated auricles with a low concentration (10^{-8}M) of propranolol altered neither the contractile force nor the rate of ^{86}Rb release. Propranolol in this low concentration possesses potent β-adrenolytic properties but does not cause the quinidine-like impairment of cardiac activity that is usually observed in higher concentrations (Kuschinsky & Rahn, 1965). In our experiments reduction of the contraction amplitude was observed at concentrations of 5×10^{-6}M or 10^{-5}M. The negative inotropic action of propranolol in the concentration range 10^{-5}–10^{-2}M was accompanied by a decrease of the ^{86}Rb efflux (Fig. 6). Thus the permeability of the membrane was reduced by propranolol in concentrations causing impairment of contraction amplitude. The dose-effect curves for the reduction in contractile force and for the impairment of ^{86}Rb release are considerably different for the concentration range investigated. Except for the highest concentrations (10^{-3} and 10^{-2}M) the propranolol effect was reversible. Only the highest concentration studied (10^{-2}M) reduced the frequency of beating (by approximately 20%).

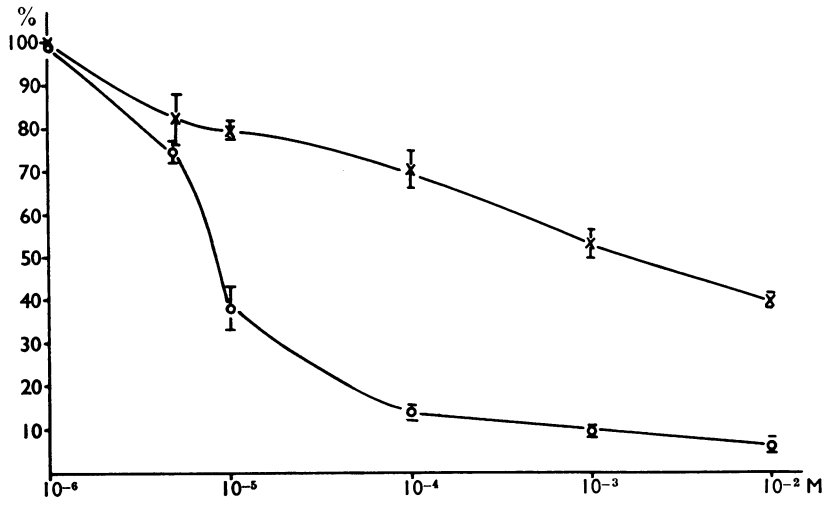

FIG. 6. Inhibition of the $^{86}Rb+$ release (×——×) and the contractile force (○——○) on exposure to propranolol. Details as described in the legend to Fig. 3.

Discussion

Although attempts have been made to relate the antiarrhythmic effect of quinidine to its impairment of the consumption of oxygen and glucose (Hess & Haugaard, 1958; for review see Conn & Luchi, 1964) it seems much more likely that the influence of this drug on ionic movements through the cell membrane is the actual cause of its antifibrillatory action. Electrophysiological studies by Lüllmann (1959) have demonstrated the retarded influx of Na^+ ions during depolarization on exposure of guinea-pig atria to quinine. In tracer experiments with ^{42}K, quinidine has been shown to reduce the K^+ efflux considerably (Holland, 1957; Holland, Klein & Briggs, 1959; Conn & Wood, 1960; Klein, Holland & Tinsley, 1960). This effect might contribute to the therapeutic action of quinidine, because according to Klein & Holland (1958) and also Conn & Luchi (1964) the occurrence of cardiac irregularities or fibrillation may be related to an increase of K^+ efflux. The experiments by Goodford & Vaughan Williams (1962), however, have demonstrated that doses of quinidine too low to affect the K^+ efflux may possess antifibrillatory activity, so the relation between the decreased ionic permeability and the antiarrhythmic effect of quinidine is not quite clear so far. Although the explanation of the antifibrillatory action of quinidine does not seem entirely satisfactory yet, the decreased permeability of the cell membrane to alkali ions appears well established. The present experiments with ^{86}Rb are in good agreement with a general reduction of membrane permeability. Qualitatively, the reduction in Rb efflux is in accordance with the inhibition of K^+ efflux by quinidine, described by other investigators (cited above). The reduced ^{86}Rb efflux was certainly not a consequence of quinidine's negative chronotropic action, because the frequency remained unchanged in the present experiments. It has already been demonstrated that a change in contraction amplitude is not accompanied by a significant alteration of the ^{86}Rb release rate. Thus calcium ions and also cardiac glycosides in non-toxic concentration greatly increased the contractile force, without significantly changing the rate of ^{86}Rb release (van Zwieten, 1968).

It is generally agreed that local anaesthetics cause a reduction in the membrane permeability for Na^+ ions during the excitation process. The present experiments have shown that tetracaine considerably diminishes ^{86}Rb efflux. This finding suggests that tetracaine also reduces the passive ion fluxes. In this respect quinidine and tetracaine seem to behave similarly. It has not been established, however, whether the antifibrillatory properties of local anaesthetics (Carden & Steinhaus, 1956; Harrison, Sprouse & Morrow, 1963; Ludena, Howard & Borland, 1963) are related to the decrease in ionic permeability caused by drugs of this type.

Hexobarbitone sodium caused a moderate impairment of the contraction amplitude, accompanied by a diminished rate of ^{86}Rb release. The latter finding is agreement with investigations by Klaus & Lüllmann (1961), who observed a reduced efflux of $^{42}K^+$ ions in guinea-pig isolated atria, exposed to hexobarbitone Na.

The unspecific, so-called quinidine-like, action of β-adrenolytic drugs in high concentrations is well known (Somani & Lum, 1965; Stanton, Kirchgessner & Parmentier, 1965; Somani, Fleming, Chan & Lum, 1966; Somani & Lum, 1966; for review see Ariens, 1967). Moreover, in the appropriate concentration β-adrenolytic drugs—for example, propranolol—show local anaesthetic activity (Howe & Shanks, 1966). In concentrations sufficient to cause quinidine-like negative inotropic effects, propranolol simultaneously inhibited the rate of ^{86}Rb release.

The present investigations with antifibrillatory agents of entirely different character have demonstrated that all the drugs studied reduced the efflux of previously accumulated $^{86}Rb^+$ ions. Previous studies have shown that $^{86}Rb^+$ can conveniently be used as a tracer for the " passive " ions in atrial tissue, especially in pharmacological experiments (van Zwieten, 1968). Concomitantly, the experiments with antifibrillatory agents and with hexobarbitone described in this paper have demonstrated that such drugs decrease the membrane permeability. The exact mechanism of this effect remains unknown, and it cannot be established whether the decrease in ionic permeability is the cause of the antifibrillatory effect of quinidine, β-adrenolytic and local anaesthetic agents.

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