Br. J. Pharmac. (1978), **63**, 427–434

THE EFFECTS OF ISOPRENALINE AND A NEW β-SYMPATHOMIMETIC AMINE UPON SPONTANEOUS ACTIVITY, DIASTOLIC DEPOLARIZATION AND PLATEAU HEIGHT IN CARDIAC PURKINJE FIBRES

W. GRABOWSKI, H.Ch. LÜTTGAU & J.J. SCHULZE

Department of Cell Physiology, Ruhr-University Bochum,
P.O. Box 102148, D-4630 Bochum 1, Germany

1 In spontaneously active Purkinje fibres of young cows the dose-response curves of the action of isoprenaline upon different electrophysiological parameters were measured.

2 The increase in slope of diastolic depolarization could roughly be described by a one-for-one binding curve with a half maximum effect near 10^{-8} M and the increase in the height of the plateau level by a two-for-one binding curve with a half maximum effect near 10^{-7} M (−)-isoprenaline.

3 These dose–response curves were similar to those of two parameters measured under voltage clamp conditions by other authors. The increase in slope of diastolic depolarization behaved like the shift of the activation curve for the pacemaker potassium current towards positive potentials and the growth in plateau height like the increase in the slow inward current mainly carried by Ca ions. From this conformity we propose that the parameters evaluated by us from action potential records could be used for a qualitative analysis of the action of catecholamines on pacemaker potassium current and Ca influx.

4 The effects of the isomers of a new drug, 1-isopropylamino-3(4'hydroxyphenoxy)-propan-2-ol (IHP), were evaluated in the same way as those of isoprenaline. The (−)-isomer was at optimal concentrations (10^{-5} M) nearly half as effective as isoprenaline in increasing frequency and slope of diastolic depolarization but caused no increase in plateau height. An identical relationship, but at 5 to 10 times higher concentrations, was obtained with the (+)-isomer.

5 When 10^{-4} M(−)-IHP was added to a preparation equilibrated with a maximum dose of (−)-isoprenaline (10^{-6} M), frequency and plateau height declined. This result together with the observation that the effects of IHP could be blocked by the specific β-antagonist propranolol, revealed the β-agonistic nature of the new drug. Its inefficiency in increasing the plateau height and thus the slow (Ca) inward current was explained by its relatively low potency and intrinsic activity.

Introduction

During recent years the effects of sympathomimetic amines upon the ion currents underlying electrical activity in cardiac pacemaker and muscle cells have been analysed in detail (cf. Noble, 1975). It is now well established that these drugs increase the slow inward current (i_{si}), mainly carried by Ca ions, without affecting its kinetics (Reuter, 1974; Reuter & Scholz, 1977a, b). Secondly, in Purkinje fibres these drugs shift the activation curve for the pacemaker potassium current (i_{K2}) in a depolarizing direction (Hauswirth, Noble & Tsien, 1968; Tsien, 1974a, b). The former effect is responsible for the increase in height of the action potential plateau and probably also for the positive inotropic action, while the latter causes an increase in the steepness of diastolic depolarization and thus an increase in frequency of activity. In addition, these drugs shorten the plateau by increasing i_{x1}, the plateau potassium current, and augment the maximum negative potential during diastole, probably by activating an electrogenic sodium pump (cf. Noble, 1975).

In the present experiments the effects of isoprenaline upon the electrical activity of Purkinje fibres were analysed together with those of the optical isomers of a new β-sympathomimetic amine, 1-isopropylamino-3(4'hydroxyphenoxy)-propan-2-ol (IHP). The alterations in the steepness of diastolic depolarization and the height of the plateau were evaluated as indirect measures of effects on i_{K2} and i_{si} respectively. The action of isoprenaline upon these parameters followed different dose-response relations. In our experiments we measured these relations simultaneously in one preparation and compared the results with those obtained after the application of the new drug IHP,

which was identified as a β-agonist of a lower potency and intrinsic activity than isoprenaline.

A preliminary account of some of the experiments described here was given at a meeting of the German Physiological Society (Schulze, 1977).

Methods

Preparation and solutions

Segments of free-running Purkinje tissue from hearts of young cows, obtained from a local slaughterhouse, were used throughout. The segments were excised within a few minutes of death and taken to the laboratory in an ice-cooled Dewar flask filled with Tyrode solution (saturated with 95% O_2 and 5% CO_2) of the following composition (mM): NaCl 137, KCl 5.4, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.42, $CaCl_2$ 5.4 and glucose 5.5. At the laboratory the segments were transferred to the experimental chamber through which a solution of the same composition gassed with 95% O_2 and 5% CO_2 was circulated (temperature 35°C). The segments were stimulated for about one hour at 0.2 Hz. After this time a modified Tyrode solution with lower concentrations of KCl (2.7 mM) and $CaCl_2$ (1.8 mM) was introduced and as a rule the segments began to beat spontaneously. After about 30 to 60 min of spontaneous beating, a microelectrode was inserted and the experiment was started. In a few experiments the resting potential was lowered by increasing the KCl concentration to 21.6 mM in the otherwise unaltered Tyrode solution with 1.8 mM $CaCl_2$. Under these conditions the Na-spike is abolished and the 'Ca action potentials' can be induced after the application of catecholamines (see p. 6).

Recording apparatus

Conventional microelectrodes of the Ling-Gerard type were used. Membrane potentials were displayed on an oscilloscope (Tektronix 561 A in combination with a Grass P18 microelectrode preamplifier) and simultaneously and continuously registered with a chart recorder (Schwarzer Varioscript 442) or a magnetic tape recorder (Bell & Howell VR 3200).

Evaluation

Evaluated were: frequency of spontaneous activity, steepness or slope of diastolic depolarization, maximum diastolic potential, maximum height of the plateau of the action potential (see Figure 1), and the threshold potential for the initiation of the regenerative action potential. The experimental data are presented as mean $\pm$ standard error of the mean (n = number of evaluations).

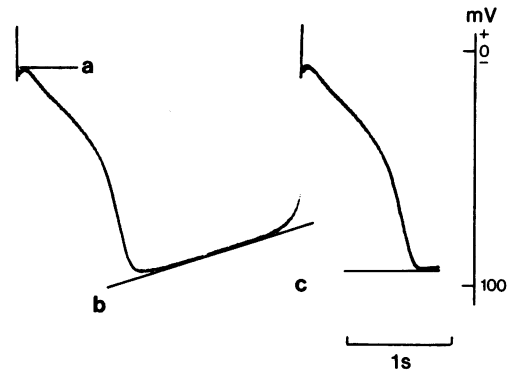


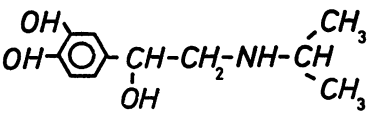
Figure 1 The evaluation of different parameters in a spontaneously beating Purkinje fibre. (a) Maximal height of the plateau phase, (b) steepness of diastolic depolarization, and (c) maximum diastolic potential.

Dose-response curves

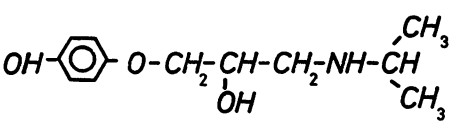
The apparent stoichiometries presented in the text ('one-for-one' binding curve for a dose-response curve with a slope of 1 in the Hill plot and 'two-for-one' binding curve for a corresponding plot with a slope of 2) do not mean to imply a direct relation between effect and drug receptor interaction. They are considered as empirical functions only, which, however, allowed a direct comparison with former voltage clamp data.

Drugs

The following sympathomimetic amines were tested: (±)-isoprenaline hydrochloride (Fluka AG); (−)-isoprenaline-(+)-bitartrate (Sigma Chemie GmbH)



and 1-isopropylamino-3(4'hydroxyphenoxy)-propan-2-ol-hydrochloride (I.H.P.) (kindly provided by Prof. H. Brunner, Ciba-Geigy AG, Basle).



The solutions of sympathomimetic amines were freshly made up during the course of the experiment. In order to retard oxidation they contained 6×10^{-4} M ascorbic acid, which itself had no effect on the

measured parameters. In the text and figures the final concentration of the drug in the bathing solution is given.

Other drugs used included: (−)-D600: α-isopropyl-α-[(N-methyl-N-homoveratryl)-γ-aminopropyl]-3,4,5-trimethoxyphenylacetonitril-hydrochloride (kindly provided by Prof. A. Oberdorf, Knoll AG, Ludwigshafen) and (±)-propranolol (kindly provided by Rhein-Pharma, Arzneimittel GmbH, Heidelberg).

Results

The effects of isoprenaline

In Figure 2 the effects of (±)-isoprenaline upon spontaneously beating Purkinje fibres are shown. At relatively low concentrations (10^{-8} M) the drug caused an increase in frequency and steepness of diastolic depolarization as well as a shift of the maximum diastolic potential in the negative direction. Higher concentrations (10^{-6} M) induced, in addition to further

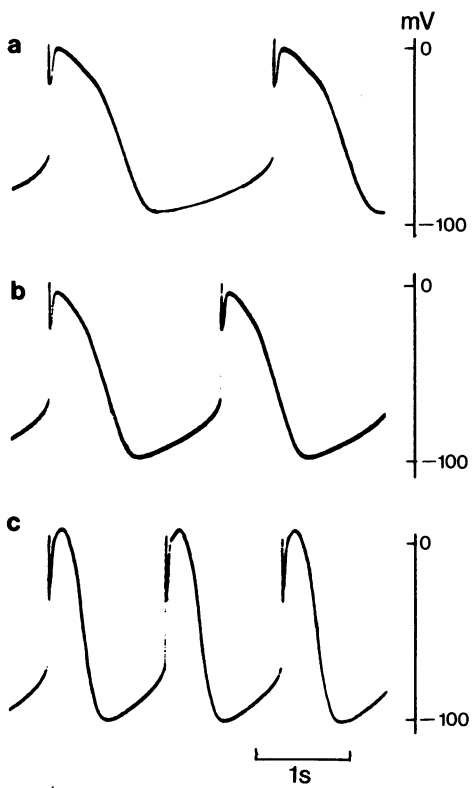


Figure 2 Action potentials of spontaneously beating Purkinje fibres before (a) and after 10 min of equilibration with 10^{-8} M (b) and 10^{-6} M (c) (±)-isoprenaline.

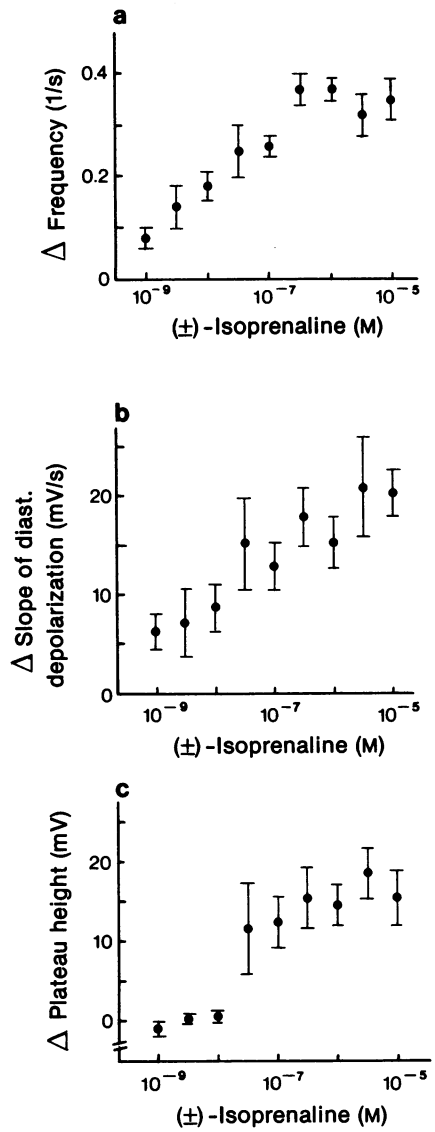


Figure 3 Dose-response curves for the extent to which (±)-isoprenaline alters (a) the frequency of the action potential, (b) the slope of the diastolic depolarization, and (c) the plateau height of the action potential. Results from 8 experiments; each point represents 4 to 11 measurements; vertical lines show s.e. means.

alterations in these parameters, an increase in the height of the plateau. The maximum effect was usually reached 5 to 10 min after application of the drug. Our results are similar to the effects of β-sympathomimetic amines described by other authors (Otsuka, 1958; Trautwein & Schmidt, 1960; Carmeliet & Vereecke, 1969; cf. Reuter, 1973). In Figure 3 the

dependence of frequency (a), slope of diastolic depolarization (b) and plateau height (c) on the concentration of (±)-isoprenaline is shown on a semilogarithmic scale. The concentration was raised from 10^{-9} to 10^{-5} M in steps of half a logarithmic unit, and the preparation remained for 12 min in each solution. It should be noted that the *alterations* (Δ) from the value under normal conditions before the application of the drug and not the absolute values (which differed considerably from preparation to preparation) were plotted on the ordinate scale. In normal Tyrode solution without drugs the following absolute values were obtained ($n = 28$): spontaneous activity 0.34 ± 0.11 Hz, slope of diastolic depolarization 11.2 ± 0.7 mV/s, maximal diastolic potential −98.5 ± 0.8 mV, threshold potential −66.2 ± 0.9 mV, maximal height of the plateau −3.8 ± 1.1 mV. All (±)-isoprenaline effects were fully reversible. However, after a high dose of the drug more than one hour was sometimes needed to regain the normal resting value.

It can be seen from Figure 3 that the first action on the frequency of the action potential and slope of diastolic depolarization was noticeable at about 10^{-9} M. The half maximal value for both effects was reached between 10^{-8} and 10^{-7} M and the maximum at 10^{-6} M. With regard to the height of the plateau phase, no alterations were observed up to 10^{-8} M. When the concentration was further increased, the maximum plateau potential shifted steeply by up to 20 mV to more positive potentials (Figure 3c). The half maximal effect was reached at about 10^{-7} M and the maximum at 10^{-6} M.

In addition to these effects, (±)-isoprenaline caused at optimal concentrations a shift of the maximum diastolic potential by about 7 to 8 mV in the negative direction (105.9 ± 2.4 mV; $n = 6$) and a slight alteration in the same direction of the threshold potential by about 3 mV (−69.5 ± 1.9 mV; $n = 6$).

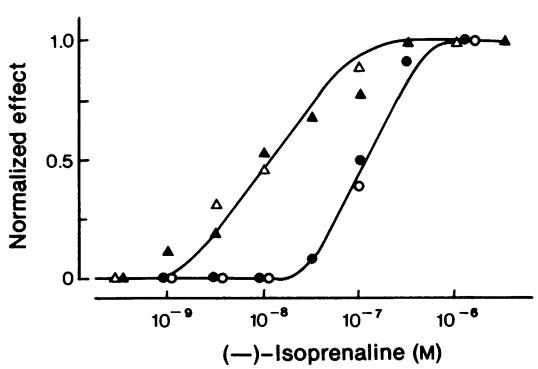


Figure 4 Normalized dose–response curves for the action of (−)-isoprenaline upon alterations in the slope of the diastolic depolarization (△, ▲) and the height of the plateau phase (○, ●). Results from two runs with the same preparation. The data were fitted by eye to a one-for-one and a two-for-one binding curve respectively.

Usually the (−)-form of a β-sympathomimetic amine is more potent than the (+)-isomer, a circumstance which may impede a stoichiometric analysis of the dose-response relation. In the experiment shown in Figure 4 and in four further experiments with similar results we avoided this difficulty by using only the (−)-isomer of isoprenaline. Apart from this alteration, the experimental procedure remained the same. It can be seen that the dose-response relation for the action of (−)-isoprenaline upon the slope of the diastolic depolarization approximates a one-for-one binding curve with a half maximal value near 1×10^{-8} M. The observed increase in steepness of diastolic depolarization is certainly related to findings by Hauswirth *et al.* (1968) who showed that under voltage clamp conditions, adrenaline shifts the activation curve for the pacemaker potassium current in the positive direction (cf. Noble, 1975). The dose-response curve for the latter effect also corresponds to a one-for-one binding curve (Tsien, 1974a). A slightly larger concentration for the half maximal effect, namely 6×10^{-8} M (Tsien, 1974a) could be explained by a higher receptor affinity for isoprenaline relative to adrenaline. Since a computer analysis (McAllister, Noble & Tsien, 1975) revealed no linear relation between the slope of diastolic depolarization and the potential shift of the activation curve for the pacemaker potassium current, additional effects of catecholamines, such as that upon the maximum repolarization in diastole, must be taken into consideration to explain the similar dose-response relationship of the two parameters. Whatever the explanation, the alterations in the steepness of diastolic depolarization may, in spite of this limitation, be regarded as a qualitative measure of the action of catecholamines on the pacemaker potassium current.

The dose-response relation for the action of (−)-isoprenaline on the plateau height, which could be fitted by a two-for-one binding curve (Figure 4), resembles the dose-response curve of the effect of (−)-noradrenaline upon the slow inward current (i_{si}) measured by Reuter (1974) under voltage clamp conditions in cat papillary muscles. Differences in the concentration for the half maximal effect between his and our measurements could again be explained by the use of different catecholamines. The Hill plot of Reuter's curve had a slope of two with a half maximal response at 5×10^{-7} M. This conformity of curves obtained by different experimental procedures was to be expected since Reuter (1974) showed in the same publication that the curve obtained from direct measurements of the slow inward current had the same concentration-response relation as that obtained from an evaluation of the plateau height (compare, in addition, Carmeliet & Vereecke, 1969). Theoreti-

cally this direct relationship can only be valid in a limited potential range of the plateau height. It will, of course, vanish when the plateau level approaches the reversal potential of i_{si}. In addition, the extent of activation of the repolarizing outward current (i_{x1}) increases with frequency, leading ultimately to a shortening and reduction of the plateau. However, under our experimental conditions only minor i_{x1}-induced changes in plateau height should occur (cf. McAllister, *et al.*, 1975). The measured rise in plateau height can therefore reasonably be regarded as a suitable measure of the increase in slow inward current.

As shown in Figure 4 a distinct difference exists between the dose-response curve for the slope of diastolic depolarization and that for the plateau height. In the following experiment we tried to find out how far the two effects are independent of each other. For this purpose we introduced the (−)-isomer of D600 which is known to block the slow inward current (see Kohlhardt, Bauer, Krause & Fleckenstein, 1972; Bayer, Kalusche, Kaufmann & Mannhold, 1975; Kass & Tsien, 1975). The preparation was first treated with 5×10^{-7} M (±)-isoprenaline, which induced the described alterations in pacemaker potential and plateau phase (Figure 5b). After 12 min (−)-D600 (1×10^{-6} M) was added to the isoprenaline-Tyrode solution (Figure 5c). It resulted in a reduction of the plateau level roughly to the value prior to the application of the drugs (Figure 5a), whereas frequency,

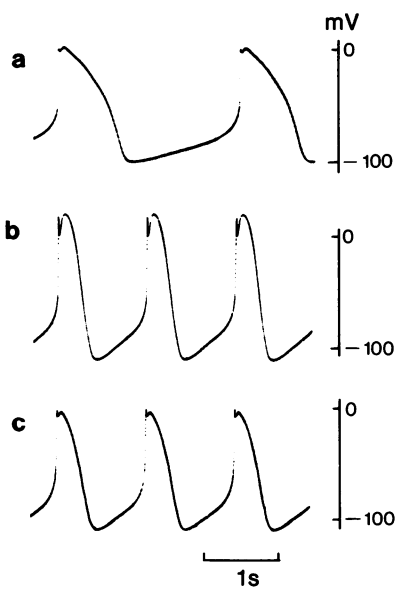


Figure 5 The effect of 5×10^{-7} M (±)-isoprenaline (b) and the same concentration of this drug plus 1×10^{-6}M (−)-D600 (c) on a spontaneously beating Purkinje fibre. (a) Normal condition before application of the drugs.

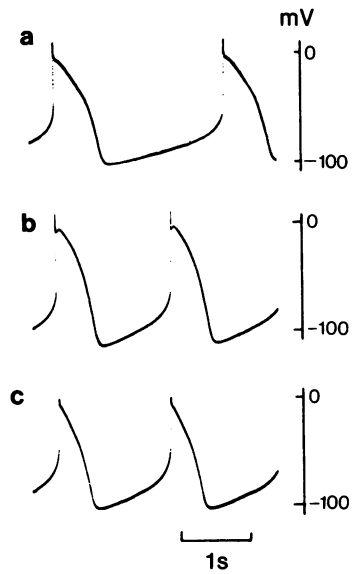


Figure 6 The effect of 1-isopropylamino-3(4′ hydroxyphenoxy)-propan-2-ol (IHP) upon the electrical activity of spontaneously beating Purkinje fibres: (a) before, (b) and (c) 10 min after the application of either 10^{-5}M of the (−)- or 10^{-4} M of the (+)-form.

slope of diastolic depolarization and duration of the action potential remained comparable to those values obtained when (±)-isoprenaline alone was present (Figure 5b). The results show that the slow inward current can be suppressed without any alterations in the kinetics of the pacemaker potassium current.

The effects of 1-isopropylamino-3(4′hydroxyphenoxy)-propan-2-ol (IHP)

The drug was applied as described for isoprenaline. In Figure 6 typical effects of the (−)-isomer (10^{-5} M) and the (+)-isomer (10^{-4} M) are shown. Both isomers increased the frequency of activity and the slope of the diastolic depolarization whilst they left the height of the plateau phase unchanged. In addition, a shift of the maximum diastolic potential towards more negative potentials was often but not always (compare Figure 6c) observed. The mean was shifted from -98.5 ± 0.8 mV to -102.8 ± 2.4 mV ($n = 5$) for the (−)-isomer (10^{-5} M) and to -103.3 ± 3.9 mV ($n = 6$) for the (+)-isomer. Practically no alteration in the threshold potential was found. The absolute value observed was -68.8 ± 3.1 mV for the (−)-isomer and -66.2 ± 3.7 mV for the (+)-isomer.

In Figure 7 the dose-response relations of the most interesting parameters are presented. It can be seen that frequency and slope of diastolic depolarization

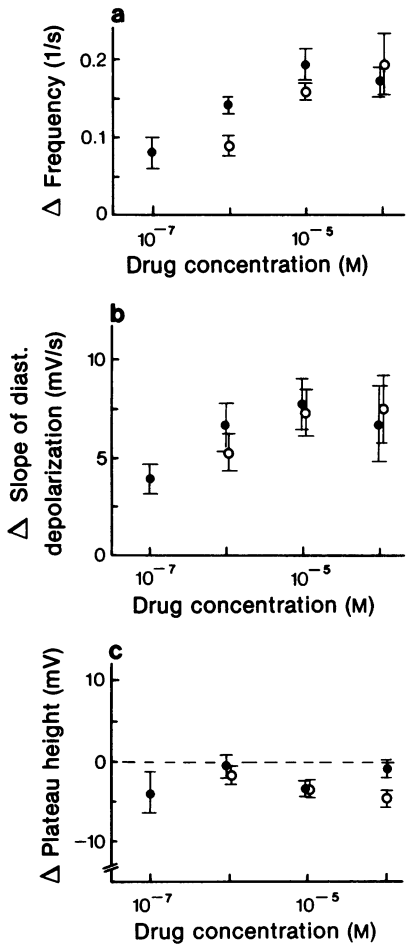


Figure 7 Dose-response curves of the changes induced by the (−)-isomer (●) and the (+)-isomer (○) of 1-isopropylamino-3(4′ hydroxyphenoxy)-propan-2-ol (IHP) upon the resting values of (a) the frequency of the action potential, (b) the slope of the diastolic depolarization, and (c) the plateau height of the action potential. Results from 6 experiments, each point represents 4 to 8 measurements; vertical lines show s.e. means.

reached a maximum value which was slightly less than 50% of that found for (±)-isoprenaline (Figure 3). The height of the plateau, however, remained roughly normal up to concentrations as high as 10^{-4} M (or even 10^{-3} M which is not shown in this figure). Very often even a small decrease was observed. The potency of IHP is also less than that of isoprenaline. First effects of the (−)-isomer on frequency and slope of diastolic depolarization could be observed at 10^{-8} to 10^{-7} M, and the maximum was reached at 10^{-5} M. With higher concentrations, frequency declined again. The dose-response curve of the (+)-isomer was similar to that of the (−)-isomer. However, as is usually observed with isomers, the potency of the (+)-form was less than that of the (−)-form. An analysis of the results from individual preparations indicated a shift of the dose-response curve for the (+)-isomer to 5 to 10 times higher concentrations.

Since IHP deviates in its action from typical β-agonists like isoprenaline, a test with a specific β-antagonist, propranolol, was made to obtain further information about its β-agonistic action. As was expected from the resemblance of the chronotropic action of IHP to that of known β-sympathomimetic amines, (±)-propranolol at a concentration of 1×10^{-6} M was sufficient to block all the described effects of both isomers of the new drug (10^{-5} and 10^{-4} M) upon spontaneously beating Purkinje fibres.

The inability of IHP to increase the plateau phase suggests that it did not cause an increase in the influx of Ca ions during the course of an action potential. This important question was further tested by repeating methods formerly described by Engstfeld, Antoni & Fleckenstein (1961) and Carmeliet & Vereecke (1969). In our experiment (Figure 8) the external KCl

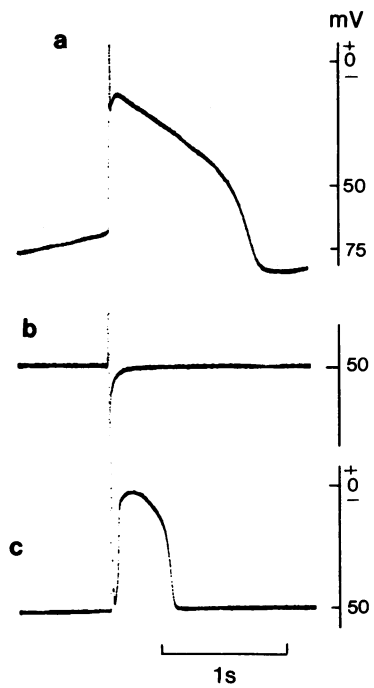


Figure 8 The effect of supramaximal stimuli upon Purkinje fibres in a Tyrode solution with 21.6 mM KCl after the addition of the (+)-form of 1-isopropylamino-3(4′ hydroxyphenoxy)-propan-2-ol (IHP) (10^{-5} M) (b) and 10^{-6} M (±)-isoprenaline (c); (a) shows a spontaneous action potential in normal Tyrode solution.

concentration was raised to 21.6 mM which caused a shift of the membrane potential to −50 mV and a blockade of spontaneous activity. The preparation was then stimulated with supramaximal stimuli. Under normal conditions and after the application of the (+)-isomer of the new drug (identical results were obtained with the (−)-isomer) only stimulation artifacts were recorded (Figure 8b). When (±)-isoprenaline at a concentration of 10^{-6} M was added 'all-or-none' Ca action potentials were induced (Figure 8c), similar to those described by the above mentioned authors. From this result it can be inferred that the new substance causes no or only a negligible increase in the influx of Ca during activity.

The agonistic action of isoprenaline and IHP

The finding that IHP increases the frequency but has no effect upon the plateau height could be explained in two ways: from its relatively low potency and intrinsic activity it could be presumed that the threshold concentration of the activator for the recruitment of additional Ca channels, i.e. cyclic adenosine 3′,5′-monophosphate (cyclic AMP, cf. Reuter & Scholz, 1977), will not be reached even with optimum concentrations of the drug. Alternatively it could be suggested that IHP is entirely without effect upon Ca channels. To decide between the two possibilities we investigated the action of (−)-isoprenaline and (−)-IHP in a qualitative form under different experimental conditions. In the first type of experiment we applied a high dose of (−)-isoprenaline (10^{-6} M). After a new steady state had been reached we added (−)-IHP at a concentration of 10^{-4} M. In four experiments the application of the second drug resulted in a decrease in the slope of diastolic depolarization by 3 to 7 mV/s and a reduction of the plateau level by 3 to 10 mV. Complementary to this experiment we induced in one preparation 'Ca action potentials' (see p. 6) with 5×10^{-6} M (−)-isoprenaline and observed a reduction of the plateau level by 6 mV after the additional application of 10^{-4} M (−)-IHP. Such a decrease is to be expected if the second agonist (−)-IHP reaches only half the intrinsic activity of the first one ((−)-isoprenaline).

In a further set of experiments we applied 10^{-8} M isoprenaline, which causes a considerable increase in the slope of diastolic depolarization but not an increase in plateau height. After equilibration we added 10^{-6} M (−)-IHP. In two experiments of this type and in several other tests with slightly different combinations of the drugs, we observed only minor alterations in both directions of the two parameters. Thus IHP alone (see Figure 7c) or in conjunction with subthreshold concentrations of isoprenaline did not cause a distinct increase in plateau height.

The experiments described in the foregoing section show that (−)-IHP reaches about 50% of the intrinsic activity of (−)-isoprenaline (slope of diastolic depolarization). In addition, the potency is two orders of magnitude lower than that of (−)-isoprenaline. If these data are applied to drug-receptor theory, the conventional theory for receptor interaction with agonist drugs (cf. Wenke, 1971) predicts qualitatively the results described in this section. In particular it can be deduced from these calculations that when (−)-IHP is added to the lower (−)-isoprenaline concentration (10^{-8} M) the 'receptor occupancy' increases, if at all, only insignificantly. Thus, the properties of (−)-IHP allowed us to demonstrate its influence upon the plateau only by its inhibitory action at relatively high concentrations of (−)-isoprenaline.

Discussion

Three results are of interest and require further comment.

The experiments described in this paper have shown that useful information about the action of catecholamines upon the pacemaker potassium current (i_{K2}) and the slow inward current (i_{si}) can be obtained from an analysis of the action potential of spontaneously beating Purkinje fibres. This is described in detail together with some limitations on p. 4. As a conclusion it can be stated that the methods applied here can be recommended for a first screening of new substances with β-sympathomimetic characteristics.

The present results confirm, for the first time in the same preparation, the divergence of the dose-response curves for the effect of catecholamines upon the pacemaker potassium current and the slow inward current. This is summarized in Figure 4 in which the action of (−)-isoprenaline upon both parameters is compared. The different slopes of the two curves, which approximate a one-for-one and a two-for-one binding curve, were the same as those found with more quantitative methods in voltage clamp experiments (Reuter, 1974; Tsien, 1974a). Assuming cyclic AMP as the common denominator of all β-adrenergic effects the divergence of the two curves is difficult to interpret. A discussion of this problem, which can be followed up in recent publications by Reuter (1974) and Tsien (1974a), is beyond the scope of the present paper.

Our experiments have shown that IHP is an agonist for the interaction with β-receptors and characterized by a low potency and intrinsic activity. At concentrations as high as 10^{-4} M it caused a considerable increase in frequency and slope of diastolic depolarization but no alterations in the height of the pla-

teau of the action potential. If the difference in the dose-response curves for the two measured parameters is similar for all catecholamines, the data presented in the Results section provide at least a formal explanation for this behaviour. In the case of isoprenaline, the plateau height began to increase when the alteration in the slope of diastolic depolarization became larger than 10 mV/s. This value was only marginally reached even with maximal effective concentrations of the new sympathomimetic amine.

The diverging dose-response relations of the parameters investigated imply that a drug with the characteristics of IHP increases frequency and slope of diastolic depolarization but remains without effect upon the plateau height, e.g. the Ca inward current during an action potential. The latter agrees with unpublished results (Lüttgau & Schulze) in which we found no increase in contractile strength in electrically stimulated (0.2 Hz) calf ventricular muscles at IHP-concentrations as high as 10^{-5} M ((−)-form) or 10^{-4} ((+)-form). In the same preparation (−)-isoprenaline (10^{-6} M) enhanced contractility by about 300%.

Finally, we would like to point out that the situation is different in atrial fibres. In a recent analysis Brown, McNaughton, Noble & Noble (1975) showed that the adrenaline-induced acceleration in pacemaker activity is ultimately caused by a large increase in a current attributable to calcium ions. The latter might explain earlier measurements by Blinks (1967) who found that the dose-response relationships for the inotropic and chronotropic effects of catecholamines are roughly identical in guinea-pig atria.

We wish to thank Professor H. Reuter, Berne, for suggesting this investigation and for helpful comments on the manuscript.

References

BAYER, R., KALUSCHE, D., KAUFMANN, R. & MANNHOLD, R. (1975). Inotropic and electrophysiological actions of verapamil and D600 in mammalian myocardium. III. Effects of the optical isomers on transmembrane action potentials. *Naunyn-Schmiedebergs Arch. Pharmac.*, **290**, 81–97.

BLINKS, J.R. (1967). Evaluation of the cardiac effects of several *beta* adrenergic blocking agents. *Ann. N.Y. Acad. Sci.*, **139**, 673–685.

BROWN, HILARY F., McNAUGHTON, P.A., NOBLE, D. & NOBLE, SUSAN J. (1975). Adrenergic control of cardiac pacemaker currents. *Phil Trans. R. Soc. Lond. B.*, **270**, 527–537.

CARMELIET, E. & VEREECKE, J. (1969). Adrenaline and the plateau phase of the cardiac action potential. Importance of Ca^{++}, Na^{+} and K^{+} conductance. *Pflügers Arch.*, **313**, 300–315.

ENGSTFELD, G., ANTONI, H. & FLECKENSTEIN, A. (1961). Die Restitution der Erregungsfortleitung und Kontraktionskraft des K^{+}-gelähmten Frosch- und Säugetiermyokards durch Adrenalin. *Pflügers Arch.*, **273**, 145–163.

HAUSWIRTH, O., NOBLE, D. & TSIEN, R.W. (1968). Adrenaline: mechanism of action on the pacemaker potential in cardiac *Purkinje* fibers. *Science (N.Y.)* **162**, 916.

KASS, R.S. & TSIEN, R.W. (1975). Multiple effects of calcium antagonists on plateau currents in cardiac *Purkinje* fibers. *J. gen. Physiol.*, **66**, 169–192.

KOHLHARDT, M., BAUER, B., KRAUSE, H. & FLECKENSTEIN, A. (1972). Differentiation of the transmembrane Na and Ca channels in mammalian cardiac fibres by the use of specific inhibitors. *Pflügers Arch.*, **335**, 309–322.

McALLISTER, R.E., NOBLE, D. & TSIEN, R.W. (1975). Reconstruction of the electrical activity of cardiac *Purkinje* fibres. *J. Physiol.*, **251**, 1–59.

NOBLE, D. (1975). *The Initiation of the Heartbeat.* pp. 1–156. Oxford: Clarendon Press.

OTSUKA, M. (1958). Die Wirkung von Adrenalin auf *Purkinje*-Fasern von Säugetierherzen. *Pflügers Arch.*, **266**, 512–517.

REUTER, H. (1973). Divalent cations as charge carriers in excitable membranes. *Progr. Biophys. molec. Biol.*, **26**, 1–43.

REUTER, H. (1974). Localization of *beta* adrenergic receptors, and effects of noradrenaline and cyclic nucleotides on action potentials, ionic currents and tension in mammalian cardiac muscle. *J. Physiol.*, **242**, 429–451.

REUTER, H. & SCHOLZ, H. (1977a). The regulation of the Ca conductance of cardiac muscle by adrenaline. *J. Physiol.*, **264**, 17–47.

REUTER, H. & SCHOLZ, H. (1977b). A study of the ion selectivity and the kinetic properties of the calcium dependent slow inward current in mammalian cardiac muscle. *J. Physiol.*, **264**, 49–62.

SCHULZE, J.J. (1977). The effects of β-sympathomimetic amines upon electrophysiological parameters in *Purkinje* fibres. *Pflügers Arch.*, **368**, R 2.

TRAUTWEIN, W. & SCHMIDT, R.F. (1960). Zur Membranwirkung des Adrenalins an der Herzmuskelfaser. *Pflügers Arch.*, **271**, 715–726.

TSIEN, R.W. (1974a). Effects of epinephrine on the pacemaker potassium current of cardiac *Purkinje* fibers. *J. gen. Physiol.*, **64**, 293–319.

TSIEN, R.W. (1974b). Mode of action of chronotropic agents in cardiac *Purkinje* fibers. Does epinephrine act by directly modifying the external surface charge? *J. gen. Physiol.*, **64**, 320–342.

WENKE, M. (1971). Drug-receptor interactions. In *Fundamentals of Biochemical Pharmacology*, ed. Bacq, Z.M., Čapek, R., Paoletti, R. & Renson, R. pp. 367–410. Oxford, New York, Toronto, Sydney, Braunschweig: Pergamon Press.

(*Received June 20, 1977.*