J. Pharm. Pharmacol. 1980, 32: 693–696

Received March 25, 1980

The effects of the optical isomers of propranolol on functional refractory period in rat isolated myocardium

D. R. TOMLINSON*, S. C. HAWORTH, N. J. HARMSWORTH AND A. M. ROSS

Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Clifton Boulevard, Nottingham, NG7 2UH, U.K.

The two optical isomers of propranolol caused a concentration-dependent increase in functional refractory period of the rat isolated left atrium. In this property the isomers were equipotent. Noradrenaline (1×10^{-7} M) decreased the functional refractory period. This effect was completely reversed by (−)-propranolol. The (+)-isomer was much less potent than (−)-propranolol in antagonizing the effects of noradrenaline on functional refractory period.

The β-adrenoceptor blocking drug propranolol also has effects on nerve and cardiac muscle characteristic of local anaesthetics (Aberg & Welin 1967; Davis 1969). These properties are manifest in the myocardium in terms of alterations in a number of variables. The antagonism of the various effects of isoprenaline (Whitsitt & Lucchesi 1967; Barrett & Cullum 1968) and of arrhythmias induced by adrenaline under halothane anaesthesia (Howe & Shanks 1966) are reflections of the β-adrenoceptor blocking effect. Direct depression of inotropy, slowing of conduction (Barrett & Cullum 1968) and increase in functional refractory period (Zetler & Strubelt 1971) are probably manifestations of the local anaesthetic or "membrane stabilizing" property of the drug. Antagonism of ouabain-induced tachycardia and arrhythmias (Howe & Shanks 1966; Barrett & Cullum 1968) is probably a reflection of both properties since ouabain releases noradrenaline as well as exerting direct effects on myocardial excitability (Govier 1965).

The (−)-isomer of propranolol has 60 to 100 times the potency of the (+)-isomer in blocking cardiac β-adrenoceptors (Howe & Shanks 1966; Barrett & Cullum 1968) whilst the two isomers are approximately equiactive in their local anaesthetic properties (Barrett & Cullum 1968).

Theoretically both β-adrenoceptor blockade and a "membrane stabilizing" effect might contribute to a prophylactic action against cardiac arrhythmias. It was therefore the objective of this study to establish and test a method for the examination of these two properties in terms of their influence on a single myocardial variable. Govier (1965) showed that the functional refractory period (FRP) of isolated, paced atria was reduced by β-adrenoceptor stimulation and Zetler & Strubelt (1971) showed that atrial FRP was increased by racaemic propranolol. Thus we set out to examine the direct effects of the optical isomers of propanolol in FRP together with their antagonism of the shortening of FRP induced by β-adrenoceptor stimulation. Isolated electrically driven left atria from rats were used throughout.

* Correspondence.

MATERIALS AND METHODS

Preparations

Left atria were removed from freshly-killed male Wistar rats (180–200 g) and suspended between parallel platinum wire electrodes in a 20 ml organ bath containing Krebs fluid (composition mM: NaCl 118, KCl 4·7, $CaCl_2$ 2·5, $MgSO_4$ 1·2, $NaHCO_3$, NaH_2PO_4 1 and glucose 11·1) gassed with 95% O_2 and 5% CO_2 at 37°C.

The atrium was connected to a Grass force-displacement transducer (FTO3C) and pre-loaded with a tension of $9{\cdot}81 \times 10^{-3}$ Newtons (1 g). The transducer was fed to a bridge-amplifier the output of which was connected to one 'Y' channel of a Tektronix D11 dual-beam storage oscilloscope.

The parallel platinum wire electrodes were connected to the S_1 channel output of a Grass S88 stimulator containing an "arrhythmic pulse" module. The atria were paced at 3Hz using 2 ms pulses. Pulse voltage was suprathreshold and was selected by determining the threshold voltage for each preparation and increasing this value by 50%. The atrium was paced in this fashion throughout the experimental protocol during which fatigue did not develop.

Measurement of the functional refractory period (FRP)

This was done according to the principle described by Govier (1965). Delivery of an extra stimulating pulse (the "arrhythmic pulse") of identical amplitude and width to the driving train will generate an extra contraction of the atrium provided that it is separated from the preceding pulse by a period of time longer than the refractory period. In practice the cells of the left atrium operate over a range of functional refractory periods and Govier (1965) selected the FRP as the minimal pulse separation time which generated a detectable second contraction of the preparation. A longer pulse separation time may be determined using, as an end-point, the generation of a contraction equal to exactly half the amplitude of the normal driven beat. Both were measured in pilot experiments and the latter ('half response time') was found to be a more reliable parameter (see 'Results)' than the former.

The extra or "arrhythmic" pulse was delivered from the S_2 channel output of the S88 stimulator and the output from both channels (i.e. all pulses applied to the electrodes) was displayed on the second beam of the oscilloscope. FRP was therefore measured from the oscilloscope screen as a "distance" between pulses. For any given steady state condition (i.e. 'resting' or propranolol alone) FRP was taken as the mean of 5 measurements. Exposure to noradrenaline gave sufficient time for only 2 or 3 measurements to be made.

Drugs

All drugs were made up in Krebs solution (see 'Preparations'). Ascorbic acid was not added to noradrenaline solutions as ascorbate "blanks" were found to alter the FRP. Noradrenaline was therefore made up freshly for each experiment and handled according to Hughes & Smith (1978).

RESULTS

Resting FRP

FRP was measured in the resting state, using both methods mentioned above, in 24 preparations. The method of Govier (1965), gave a mean value of 115·0 ms with a s.e.m. of 6·3 (5·5% of the mean). That using a half-normal response gave a mean value of 151·7 ms with a s.e.m. of 2·2 (1·4% of the mean). As the "half-response time" was considered to be more reliable and must reflect the FRP of a greater proportion of the contractile cells of the atrium, it was adopted for studies on the actions of drugs.

Effects of tetrodotoxin

Field stimulation of the left atrium in vitro with the pulse amplitudes adopted should not excite intramural autonomic nerves leading to noradrenaline or acetylcholine release. Stimuli in excess of 10 V were not used (with similar apparatus and pulse widths, nerve excitation threshold is in excess of 40 V for rat isolated left atria, Bennett et al 1976). Pilot experiments on 4 preparations showed 1×10^{-6} M tetrodotoxin had no effect on FRP and it may be inferred that the stimulus parameters we used do not cause release of autonomic transmitters.

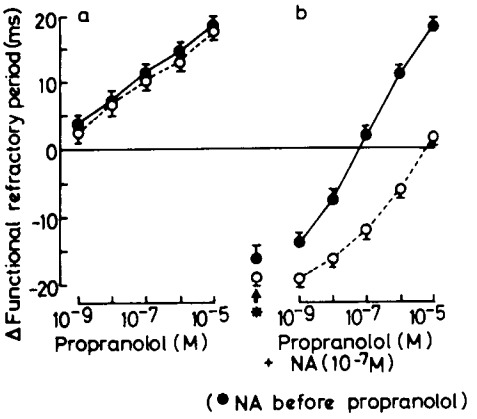

FIG. 1 (a) shows the direct effects of (+)-propranolol (○, n = 12) and (−)-propranolol (●, n = 16) on functional refractory period in rat isolated left atria. In the centre the responses of two separate groups of preparations to noradrenaline (NA 1×10^{-7} M) are shown as controls for 1(b) which shows the effects of the (+)-isomer (○, n = 9) and of the (−)-isomer (●, n = 10) on FRP in the presence of noradrenaline. Limits denote 1 s.e.m.

Direct effects of the optical isomers of propranolol

The effects of progressively increasing concentrations (from 10^{-10} to 10^{-5} M) of (+)- and (−)-propranolol on FRP were studied in 12 and 16 preparations respectively, FRP being measured between 8 and 10 min after establishment of each concentration of each drug.

Neither isomer had any significant effect at 10^{-10} M. Fig. 1a shows that from 10^{-9} to 10^{-5} M both isomers produced a progressive and linear increase in FRP. At 10^{-5} M the (+)-isomer produced an increase of 17·6 ± 1·0 (s.e.m.) ms and the (−)-isomer an increase of 18·7 ± 1·1 (s.e.m.) ms. The difference was not significant.

Effects of noradrenaline

Pilot experiments having shown that noradrenaline (NA) caused a concentration-dependent decrease in FRP, 1×10^{-7} M was selected as a reference concentration because its effects on rate in vitro (a tachycardia of approximately 100 beats min^{-1}) are within physiological dimensions and because its effect on FRP lay on the linear portion of the concentration-effect plot.

The effect of NA on FRP was measured between 1 and 2 min after addition of the drug to the bath. During this period FRP and force of contraction remained steady.

In two separate groups of left atria NA (1×10^{-7}M) produced a decrease in FRP of 16·0 ± 1·8 (s.e.m., n = 10) ms and 18·3 ± 0·8 (s.e.m., n = 9) ms (see Fig. 1). These responses of the two groups were not significantly different (unpaired *t*-test).

Effects of propranolol isomers with noradrenaline

Propranolol was first added to the bath followed, 8 min later, by 1×10^{-7} M NA, 1–2 min after which FRP measurements were made. (−)-Propranolol at 10^{-9} M slightly decreased the effect of NA on FRP (ΔFRP = 14·0 ± 1·6 (s.e.m., n = 10), $P < 0.01$ by paired *t*-test) (Fig. 1). Further increments in the (−)-isomer progressively reversed the effect of NA culminating in an increase in FRP at 10^{-5} (−)-propranolol which was almost identical to that seen previously with the (−)-isomer alone at 10^{-5} M.

The (+)-isomer at 10^{-9} M had no effect on the response to NA. Increasing concentrations of the (+)-isomer produced a progressive attenuation of the decrease in FRP seen previously with NA alone. In the presence of 10^{-7} M NA the relationship between ΔFRP and (+)-propranolol concentration was non-linear (Fig. 1b).

DISCUSSION

A method derived from that of Govier (1965) has been employed to measure FRP in rat isolated left atria. The end point selected was the temporal separation of two suprathreshold stimulating pulses which gave a contraction, in response to the second pulse, of exactly half the normal amplitude. The delay thus measured probably reflects something close to the average FRP for the preparation though, for a given preparation, the range from the shortest to the longest FRP is 70 to 80 ms. At the present level of understanding, the authenticity and validity of the chosen parameter as an index of FRP can be evaluated only pragmatically. The "half-response time" was easily and exactly measured on the storage oscilloscope. The scatter for a group of preparations was small as were the standard errors on all means. The parameter therefore permits the measurement of drug-induced changes with high resolution.

The nature of the measurement dictates that it may be frustrated by inotropic changes in muscle performance. The higher concentrations of both isomers of propranolol reduced force of contraction and noradrenaline caused increases in inotropy. For these reasons it was considered vital that FRP was measured in conditions as close to steady state as possible. In pilot experiments changes in force of contraction induced by altering the pre-load applied to the muscle did not cause significant changes in FRP. Under the conditions of our measurements it is highly unlikely that inotropic influences could change during the period separating the normal and attendant "arrhythmic" pulse. We would, therefore, propose that the parameter was not influenced by drug-induced changes in force of contraction.

The salient outcome of this investigation is of a methodological nature in that, in a single test preparation, both of the properties of propranolol which give rise to its antiarrhythmic action have been evaluated (Fig. 1).

The direct effects of isomers on the myocardium are manifest as a concentration-dependent increase in FRP (Fig. 1a). This property resembles that of lignocaine and may be related to a drug-induced delay in reactivation of the Na^+ system of the cardiac muscle cell (Iven & Brasch 1977).

The regression lines in Fig. 1b reflect a combination of the direct effects on FRP together with antagonism, increasing progressively with propranolol concentration, of the shortening of FRP caused by noradrenaline. The plot for the (−)-isomer indicates that β-adrenoceptor blockade commences between 10^{-9} and 10^{-8} M. At 10^{-5} M the (−)-isomer had blocked completely the effects of noradrenaline and the increased FRP was almost identical to that seen when the drug was used without catecholamine (Fig. 1a). If (+)-propranolol had no β-adrenoceptor blocking property the concentration/response plot of its effects in the presence of noradrenaline would be expected to have the same slope as that shown in Fig. 1a but to show a downward displacement on the ordinate by an amount equal to the fall in FRP produced by 10^{-7} M noradrenaline. In fact the slope between 10^{-9} and 10^{-8} M shows these characteristics indicating an absence of β-adrenoceptor blockade. Further increases in (+)-isomer

concentration gave a progressive increase in the gradient of the plot reflecting the development of β-adrenoceptor blockade. Thus the results support earlier evidence that the (+)-isomer is markedly less potent than the (−)-isomer as a β-adrenoceptor blocker (Howe & Shanks 1966; Whitsitt & Lucchesi 1967; Barrett & Callum 1968).

It is generally agreed that the clinically effective antiarrhythmic drugs studied so far share the property of acting directly on the myocardium to increase FRP (Zetler & Strubelt 1971). Thus, even though the precise details of such pharmacological effects and the pathogenesis of arrhythmias are not fully understood (Pamintuan et al 1970), the technique reported here would appear to have sufficient predictive value to be of worth in the evaluation of new compounds. Clearly this would be particularly true of compounds which combine β-adrenoceptor blocking and local anaesthetic actions.

Acknowledgements

We would like to thank ICI Ltd for their generous gifts of (+)- and (−)-propranolol. We are indebted to Mrs Ann White for her excellent technical assistance.

REFERENCES

Aberg, G., Welin, I. (1967) Life Sci. 6: 975–979

Barrett, A. M., Cullum, V. A. (1968) Br. J. Pharmacol. 34: 43–55

Bennett, T., Gardiner, S. M., Tomlinson, D. R. (1976) Naunyn-Schmiedeberg's Arch. Pharmakol. 293: 175–182

Davis, W. G. (1969) J. Pharm. Pharmacol. 22: 284–290

Govier, W. C. (1965) J. Pharmacol. Exp. Ther. 148: 100–105

Howe, R., Shanks, R. G. (1966) Nature (London) 210: 1336–1338

Hughes, I. E., Smith, J. A. (1978) J. Pharm. Pharmacol. 30: 124–125

Iven, H., Brasch, H. (1977) Naunyn-Schmiedeberg's Arch. Pharmakol. 297: 153–161

Pamintuan, J. C., Dreifus, L. S., Watanabe, Y. (1970) Am. J. Cardiol. 26: 512–519

Whitsitt, L. S., Lucchesi, B. R. (1967) Life Sci. 6: 939–950

Zetler, G., Strubelt, O. (1971) Naunyn-Schmiedeberg's Arch. Pharmakol. 271: 335–345