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The reversal of experimental cardiac arrhythmias by indoramin (Wy 21901)

B. J. ALPS, MADELINE HILL, K. FIDLER, E. S. JOHNSON AND A. B. WILSON

Department of Pharmacology, Wyeth Institute of Medical Research, Taplow, Bucks, U.K

Indoramin was approximately equipotent with (±)-propranolol but more potent than lignocaine in reversing adrenaline-induced multiple ventricular ectopic beats and ventricular tachycardia in halothane-anaesthetized cats. Indoramin was more potent than either propranolol or lignocaine in reversing ouabain-induced ventricular tachycardia or fibrillation to sinus rhythm. The local anaesthetic activity of indoramin was three times greater than that of procaine and lignocaine and twice that of (±)-propranol and quinidine when assessed by the guinea-pig weal test. Experiments on the guinea-pig isolated ileum supporting a local anaesthetic action of indoramin showed that nicotine contractions were reduced by indoramin whereas acetylcholine responses were unaffected. Based on the evidence presented and that published elsewhere, it is considered likely that indoramin abolishes adrenaline-induced arrhythmias by a combination of membrane stabilization and blockade of myocardial α-adrenoceptors whereas ouabain arrhythmias may be reversed by membrane stabilization alone.

Indoramin (Wy 21901) is a potent hypotensive drug which possesses α-adrenoceptor blocking, myocardial inhibitory and anti-arrhythmic properties (Alps, Hill & others, 1970; Alps, Johnson & Wilson, 1970). Since these authors have shown the cardiac actions of indoramin not to be due to β-adrenoceptor blockade, experiments have been made to determine the mechanism of the anti-arrhythmic action of the drug.

METHODS

Local anaesthetic evaluation

Local anaesthesia was measured by the guinea-pig weal test (Bülbring & Wajda, 1945) and from the selective depression of contractions of the guinea-pig isolated ileum induced by nicotine.

A mean percentage response, with standard errors, for each concentration of drug was obtained from the results of 4–6 injections of 0·25 ml of test solution given intradermally into the anterior and posterior areas of the shaved flanks of guinea-pigs (>550 g). Saline was used as a control. A log dose-response curve was then plotted to give an estimate of the degree of local anaesthesia produced over the 30 min experimental period as assessed every 5 min by pricking the weal.

Test compounds, in 0·9% saline, were examined over the following concentration ranges: indoramin hydrochloride, $2{\cdot}5 \times 10^{-4}$ to 5×10^{-3}M (its solubility limit in saline); lignocaine hydrochloride, 10^{-3} to 2×10^{-2}M; (±)-propranolol hydrochloride, 10^{-3} to 2×10^{-2}M; procaine, $2{\cdot}65 \times 10^{-3}$ to $8{\cdot}45 \times 10^{-2}$M; quinidine sulphate, 10^{-3} to 10^{-2}M.

Nicotine contractions of the guinea-pig ileum. Guinea-pig ileum, in 2 cm segments, was set up as described by Brownlee & Johnson (1963). Agonist-antagonist experi-

ments were made and agonist dose-response curves plotted to nicotine and acetylcholine, alone and in the presence of indoramin hydrochloride (10^{-7} to 10^{-5}M). The recovery of the responses was followed after washing in antagonist-free Krebs solution.

Anti-arrhythmic evaluation

Adrenaline-induced arrhythmias. Cats of either sex (2–4 kg) were anaesthetized with 1–2% halothane in oxygen. Aortic, carotid or left ventricular blood pressure was measured by means of a Statham P23 pressure transducer via a catheter inserted through the left carotid artery. E.c.g. recordings were made from bipolar and augmented unipolar limb leads, although throughout the experiment lead II was the standard reference lead; the other leads were used to detect the earliest return from arrhythmias to sinus rhythm when lead II records were equivocal. Most e.c.g.'s were recorded on a Mingograf Cardirex 24 ink spray oscillograph, a few were recorded on a Grass model 7 polygraph.

Adrenaline was infused continually from a motor-driven syringe through a catheter in the right femoral vein. The rate of infusion was generally 3 μg/kg min^{-1} or less but in some cases higher rates were used to induce severe arrhythmias.

The anti-arrhythmic test compounds were injected through a catheter in the right cephalic vein.

Ouabain-induced arrhythmias. Cats were anaesthetized with pentobarbitone sodium (30 mg/kg intrapleurally). Blood pressure and e.c.g. were recorded as before. Glycoside arrhythmias were induced by the intravenous injection of ouabain, 60 μg/kg initially, followed 30 min later by doses of 10 μg/kg every 10 min until the required severity of arrhythmia was produced.

Anti-arrhythmic test substances were injected intravenously either during the development of severe arrhythmias or immediately after the onset of ventricular fibrillation.

RESULTS

Local anaesthesia

Guinea-pig weal. Log dose-response curves for indoramin, (±)-propranolol and procaine for the 30 min experimental period were parallel and linear over the intermediate range (Fig. 1). The points for lignocaine fell either side of the curve for procaine and the

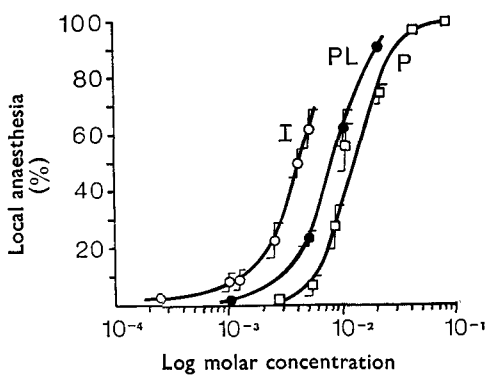

FIG. 1. Log dose-response curves to show % local anaesthesia produced by indoramin hydrochloride (I), propranolol hydrochloride (PL) and procaine (P), as assessed over a 30 min period by the guinea-pig weal method. The points for lignocaine hydrochloride fell either side of the curve for procaine; the points for quinidine sulphate were almost identical with those for propranolol.

points for quinidine sulphate were almost identical with those for propranolol. The relative potencies estimated at the ED50 level of these curves were:—

Indoramin > propranolol = quinidine > lignocaine = procaine
3·1 : 1·6 : 1·7 : 1 : 1

The degree of local anaesthesia estimated over the 30 min for indoramin at its maximum solubility in 0·9% saline at room temperature (5×10^{-3}M) was only 62%. The remaining drugs were sufficiently soluble to enable the use of higher concentrations and the production of complete anaesthesia throughout the 30 min observation period.

Guinea-pig isolated ileum. Nicotine dose-response curves were unaffected by concentrations of indoramin hydrochloride of 10^{-6}M and less. However, concentrations of 5×10^{-6} and 10^{-5}M reduced the nicotine responses, so that the maximal response was only 20% of the control maximal response to acetylcholine (Fig. 2A, B).

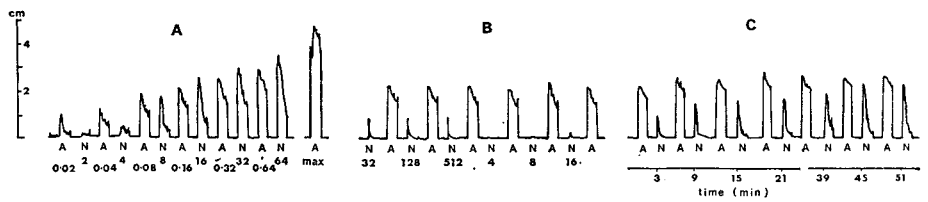

FIG. 2. Contractions of the guinea-pig isolated ileum to alternate doses of acetylcholine (A) and nicotine (N); the doses are μg base added to a 12 ml bath. (A) Control dose-response curves. (B) Dose-response curve for nicotine and responses to constant doses of acetylcholine (0·16 μg) after 60 min treatment with 10^{-5}M indoramin hydrochloride. (C) Responses to constant doses of acetylcholine (0·16 μg) and nicotine (16 μg) showing the progressive recovery of the nicotine response in antagonist-free Krebs solution. In C the recorder was stopped for 12 min before the 39 min point.

The block produced by 5×10^{-6} and 10^{-5}M indoramin hydrochloride was completely reversed within 16 and 45 min respectively by washing in antagonist-free Krebs solution (Fig. 2C). Acetylcholine dose-response curves were unaffected by these concentrations of indoramin.

Thus, on this preparation indoramin antagonized responses to nicotine in concentrations that had no effect on responses to acetylcholine.

Anti-arrhythmic tests

Adrenaline arrhythmias were easy to maintain and would generally reverse within a few minutes of stopping the infusion. The induction dose varied; rates as low as 0·6 μg/kg min^{-1} were occasionally successful. The normal dose used (3 μg/kg min^{-1}) caused multiple atrial or ventricular extrasystoles, or ventricular tachycardia; ventricular fibrillation was induced on only a few occasions. After effective doses of some of the anti-arrhythmic drugs the animal could withstand increases in the adrenaline infusion to 60 μg/kg min^{-1} without cardiac failure.

Ouabain-induced arrhythmias were much more severe than those caused by adrenaline and were of sudden onset. They usually resulted in ventricular fibrillation. The usual total cumulative dose before arrhythmias were seen was between 80–90 μg/kg. Usually the anti-arrhythmic drugs were tested against arrhythmias caused by low doses of ouabain and then again against ventricular fibrillation induced by

larger doses. Sometimes the glycoside induced fibrillation early in the experiment, then the drugs were tested against fibrillation only.

Adrenaline arrhythmias

Indoramin. Indoramin reversed multiple ventricular extrasystoles caused by adrenaline 3 μg/kg min^{-1} and at doses lower than those of lignocaine. For example, in one cat indoramin, 28 μg/kg, abolished the extrasystoles for 200 s (Fig. 3) and in

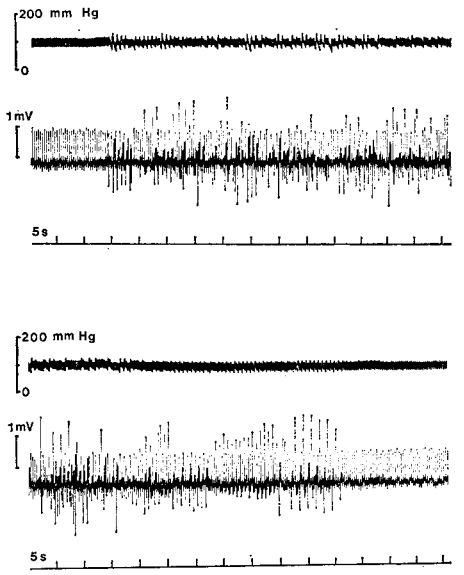

FIG. 3. Records of the left carotid blood pressure and lead II of the e.c.g. in a halothane-anaesthetized cat. In the Lh panel multiple ventricular extrasystoles have been induced by a constant intravenous infusion of 3 μg/kg min^{-1} of adrenaline. In the Rh panel 28 μg/kg indoramin hydrochloride re-established sinus rhythm within 58 s. Normal rhythm was maintained for 200 s before multiple ventricular extrasystoles recurred in response to the continuous adrenaline infusion. The interval between the panels was 100 s.

most animals 0·1 mg/kg reversed the gross ectopic beats caused by 6 μg/kg min^{-1} adrenaline. Indoramin, 1 mg/kg, reversed adrenaline-induced arrhythmias in all five animals tested.

Propranolol. The anti-arrhythmic activity of propranolol was similar to that of indoramin. In one experiment, multiple ventricular extrasystoles caused by adrenaline (3 μg/kg min^{-1}) were reversed to sinus rhythm by 10 μg/kg propranolol. In two other cats, 0·1 mg/kg propranolol successfully reversed the multiple ventricular extrasystoles (Fig. 4); 1 mg/kg propranolol reversed these arrhythmias in all animals and a marked increase in the adrenaline infusion rate ($\times 5$) was required to re-initiate the abnormal rhythm.

High doses of propranolol (>3 mg/kg) complicated the experiment by inducing conduction changes, and in one cat 3·6 mg/kg propranolol caused complete electrical silence. In contrast, indoramin failed to cause similar effects when given in repeated doses up to 15 mg/kg.

The occurrence of adrenaline-induced ventricular fibrillation is relatively uncommon and was reversed by propranolol in one out of 2 cases.

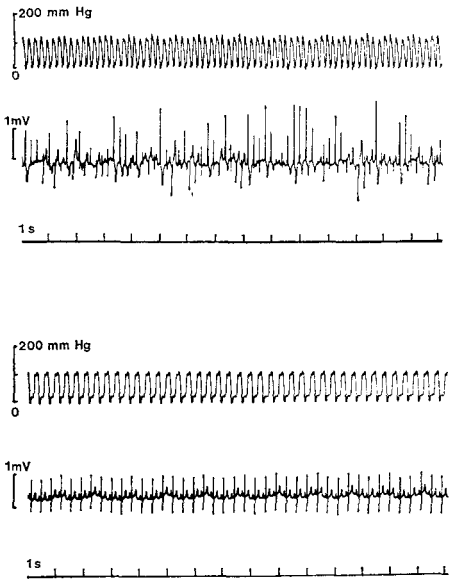

FIG. 4. Records of left intraventricular pressure and lead II of the e.c.g. in a halothane-anaesthetized cat. In the Lh panel a ventricular arrhythmia, characterized by multiple extrasystoles, was induced by a constant intravenous infusion of 3 μg/kg min^{-1} of adrenaline. In the Rh panel 0·1 mg/kg propranolol re-established sinus rhythm within 20 s (recorded 44 s after the start of injection). Normal rhythm was maintained for 15 min before the ventricular arrhythmia recurred in response to the continuous adrenaline infusion. The interval between the panels was 100 s.

Lignocaine. The dose of lignocaine required to reverse adrenaline-induced multiple ventricular extrasystoles varied widely (0·4–5 mg/kg) from animal to animal and the arrhythmia reverted to sinus rhythm in only 2 out of 3 experiments. However, the gross arrhythmia in the third animal was partially reversed by 3·2 mg/kg indicating considerable stabilization of the myocardium.

Ouabain arrhythmias

Indoramin. Ouabain-induced ventricular tachycardia (ouabain dose 80–140 μg/kg) was readily reversed to sinus rhythm by indoramin (0·25–2 mg/kg) in all 4 experiments made (Fig. 5). Ouabain-induced ventricular fibrillation was also reversed by indoramin (dose 1–2 mg/kg) in 4 out of 4 experiments (Fig. 6).

Propranolol. Ouabain-induced coupling, multiple extrasystoles and ventricular tachycardia (ouabain dose 76–160 μg/kg) were converted to sinus rhythm by propranolol (mean 5 mg/kg) in 2 out of 3 animals. Fig. 7 shows a ventricular tachycardia caused by 120 μg/kg ouabain which was reversed to sinus rhythm by propranolol (1·1 mg/kg). In the third animal grossly abnormal ventricular complexes were not reversed to sinus rhythm by 7 mg/kg propranolol but, as judged from the blood pressure response, the contractions of the myocardium were more co-ordinated and mechanically effective.

Ouabain-induced ventricular fibrillation (1 experiment) was not reversed by propranolol (4 mg/kg).

Lignocaine. Ouabain-induced coupling and ventricular extrasystoles (ouabain dose 90–156 μg/kg) were reversed by lignocaine in doses of 1–2 mg/kg. Lignocaine

(4 mg/kg) failed to reverse ouabain-induced ventricular fibrillation in the 2 experiments in which this arrhythmia was studied.

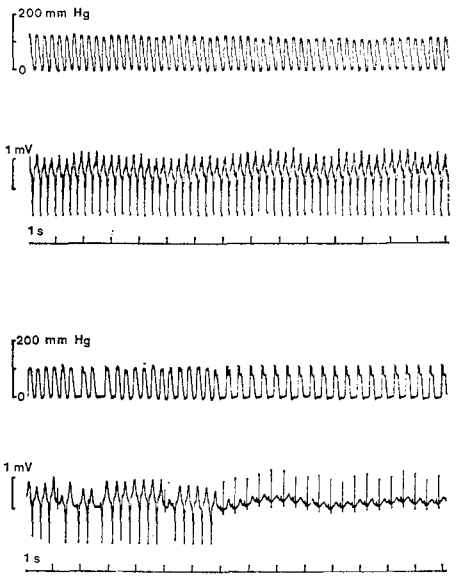

FIG. 5. Records of left intraventricular pressure and lead II of the e.c.g. in a pentobarbitone-anaesthetized cat. The Lh panel shows a ventricular tachycardia induced by a cumulative dose of 100 μg/kg of ouabain. In the Rh panel 2 mg/kg indoramin hydrochloride re-established sinus rhythm within 50 s (recorded 40 s after the start of injection). Normal rhythm was maintained for 12 min before ventricular tachycardia recurred. The interval between the upper and lower records was 60 s.

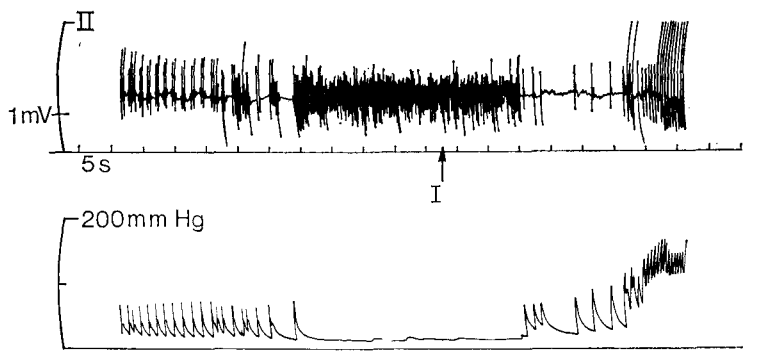

FIG. 6. Lh tracing is lead II of the e.c.g. after a cumulative dose of 80 μg/kg of ouabain, showing characteristic coupling and ventricular ectopic beats leading on to ventricular fibrillation. The Rh tracing shows the aortic blood pressure recorded simultaneously with the e.c.g. It can be seen that an effective myocardial output was restored within 15 s of the intravenous injection of indoramin (1 mg/kg) accompanied by partial reversal to normal rhythm.

DISCUSSION

Indoramin (Wy 21901) has been shown to be an effective anti-arrhythmic agent active against both adrenaline-induced and ouabain-induced arrhythmias. Indoramin was approximately equipotent with propranolol but more potent than lignocaine in reversing adrenaline-induced multiple ventricular ectopic beats and ventricular

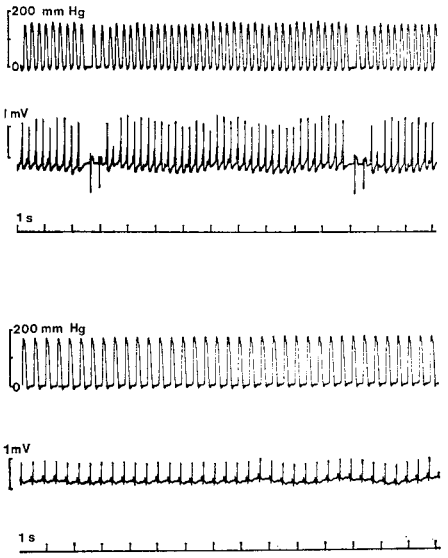

FIG. 7. Records of left intraventricular pressure and lead II of the e.c.g. in a pentobarbitone-anaesthetized cat. The Lh panel shows a ventricular tachycardia induced by a cumulative dose of 120 μg/kg of ouabain. In the Rh panel 1·1 mg/kg propranolol re-established sinus rhythm within 40 s (recorded 65 s after the start of injection). Normal rhythm was maintained for 10 min with occasional ectopic beats occurring up to 16 min before ventricular tachycardia recurred. The interval between records was 90 s.

tachycardia in halothane-anaesthetized cats. In contrast, indoramin was superior to both propranolol and lignocaine in opposing ouabain-induced ectopic beats, ventricular tachycardia or ventricular fibrillation in pentobarbitone-anaesthetized cats.

Although indoramin and the β-adrenoceptor blocking agent propranolol were equally effective in reversing adrenaline-induced arrhythmias, blockade of cardiac β-adrenoceptors cannot explain the anti-arrhythmic properties of indoramin. Evidence for this conclusion is given by the work of Alps & others (1970) who showed that even in high concentrations, indoramin was without β-adrenoceptor blocking activity against the relaxation of the guinea-pig tracheal spiral induced by noradrenaline and isoprenaline, or the increase in the force and rate of contraction of the rabbit isolated heart caused by isoprenaline.

It has been known for some 20 years that α-adrenoceptor blocking agents effectively inhibit cardiac arrhythmias induced by adrenergic stimuli (Acheson, Farah & French, 1949; Nickerson & Nomaguchi, 1951), but only recently has interest in this area been renewed (Brkic & Stern, 1965; Garvey, 1969). Garvey (1969) and Nickerson & Hollenberg (1967) have provided evidence that the cardiac adrenergic receptors responsible for these arrhythmias are α-adrenoceptors that are selectively blocked by α-adrenoceptor blocking drugs. It seems probable that at least part of the anti-arrhythmic action of indoramin is due to its potent α-blocking action ($pA_2 = 7{\cdot}4$; Alps & others, 1970) on the α-adrenoceptors in the myocardium.

Blockade of α-adrenoceptors in the peripheral vasculature may also contribute to the cardiac anti-arrhythmic actions of indoramin. Lees & Tavernor (1970), Dresel (1962), Riker, Depierre & others (1955), Roberts, Standaert & others (1956) have shown that in the horse, cat and dog, adrenaline induces arrhythmias both by an

adrenergic action on the myocardium and also indirectly by an increase in vagal tone. This vagal effect is probably a reflex response to the pressor action of adrenaline and can be expected to be antagonized by an α-adrenoceptor blocking drug such as indoramin, which produces a significant lowering of systemic blood pressure (Alps, Johnson & Wilson, 1970).

Because of the known local anaesthetic activity of propranolol (Davis, 1970), and because of the similarity in the cardio-inhibitory actions of propranolol and indoramin in the rabbit isolated heart (Alps & others, 1970) and the anaesthetized cat (Alps, Johnson & Wilson, 1970), experiments were made to determine the local anaesthetic potency of indoramin. By the guinea-pig weal method, its action was found to be three times greater than that of procaine and lignocaine and twice that of propranolol and quinidine. Confirmation of this property of indoramin was supplied by experiments with the guinea-pig isolated ileum in which responses to nicotine were antagonized by the drug while those to acetylcholine were unaffected. Since nicotine contracts the ileum by an indirect action on the cholinergic innervation and acetylcholine acts directly on the smooth muscle, the most likely site of action of indoramin is on the nerve fibres of the intramural plexus.

Local anaesthetic drugs are extremely effective in stabilizing the myocardial membrane, causing reduction of depolarization and depression of irritable foci. Because local anaesthetic membrane stabilization is non-specific, all irritable foci will be modified regardless of their aetiology. Thus the local anaesthetic drug, lignocaine, was approximately equiactive in reversing both adrenaline- and ouabain-induced arrhythmias. The greater potencies of propranolol and indoramin in antagonizing adrenaline-induced arrhythmias would appear to be explained by their additional properties of adrenoceptor-blockade. In contrast, ouabain-induced arrhythmias are not known to be associated with myocardial adrenoceptors and it is to be anticipated that anti-arrhythmic activity against ouabain will be related to membrane stabilizing or local anaesthetic potency of the anti-arrhythmic drugs. This relation is borne out by the present experiments which showed indoramin to be superior either to propranolol or lignocaine in opposing ouabain-induced arrhythmias, the order of anti-arrhythmic activity being the same as the local anaesthetic order of potency of indoramin, 3·1, (±)-propranolol, 1·6 and lignocaine, 1·0, as assessed on the guinea-pig skin by the Bülbring and Wajda technique.

In conclusion it seems likely that indoramin abolishes adrenaline-induced arrhythmias by virtue of its α-adrenoceptor blocking and membrane stabilizing actions, whereas ouabain-induced arrhythmias may be antagonized by membrane stabilization alone.

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