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Quantitative analysis on isolated organs of the autonomic blocking properties of indoramin hydrochloride (Wy 21901)

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

Department of Pharmacology, Wyeth Institute of Medical Research, Taplow, Bucks

Summary

1. Indoramin is a competitive α-adrenoceptor blocking agent on the guinea-pig isolated vas deferens and aortic strip; the pA_2 value on the aorta is 7·4.

2. Indoramin is devoid of β-adrenoceptor blocking activity on the guinea-pig isolated trachea (10^{-4}M) and the Langendorff preparation of the rabbit heart (10^{-6}M). It also has no adrenergic neurone blocking action on the Finkleman preparation of the rabbit small intestine (10^{-5}M).

3. On the Langendorff preparation of the rabbit isolated heart, indoramin (above 10^{-6}M) has cardio-inhibitory properties similar to those of propranolol.

4. On the guinea-pig isolated ileum (10^{-5}M) and trachea (10^{-6}M), indoramin is devoid of anticholinergic activity, but has a potent antihistamine action which satisfies the criteria for competitive antagonism; the pA_2 value for this antagonism on the ileum is 8·2.

5. Indoramin antagonizes 5-hydroxytryptamine on the rat isolated fundus and ileum; the pA_2 value for the antagonism on the ileum is 6·0.

Introduction

Indoramin hydrochloride (3-[2-(4-benzamidopiperid-1-yl)ethyl] indole hydrochloride, Wy 21901) is a potent hypotensive compound (Archibald, 1968) which lowers blood pressure in conscious or anaesthetized, normotensive or hypertensive animals, whether given by oral or parenteral administration (Alps, Johnson & Wilson, 1970c; Alps, Archibald, Johnson & Wilson, 1970a).

This paper describes the actions of indoramin on isolated organ preparations. Part of this work has been communicated to the British Pharmacological Society (Alps, Hill, Johnson & Wilson, 1970b).

Methods

All experiments on isolated organs were made using Krebs solution maintained at 37° C and bubbled with 5% carbon dioxide in oxygen. Organ bath volumes of 10 ml were used except for experiments on the guinea-pig aortic strip and tracheal spiral where the bath volume was 20 ml. In all experiments with antagonists,

* Present address: Department of Health and Social Security, Medicines Division, Finsbury Square House, 33–37a Finsbury Square, London EC2A 1PP.

equilibrium with the tissue was judged to have occurred when constant agonist responses were elicited. Where appropriate, ascorbic acid ($5{\cdot}68 \times 10^{-5}$M) was added to prevent oxidation of noradrenaline.

Guinea-pig vas deferens

(See Leach, 1956.) Contractions were recorded on smoked paper by isotonic frontal writing levers (load 0·75 g; 7-fold magnification). Doses of noradrenaline were added for a contact time of 1 min in each 5 min period and washed out 3 times by overflow.

Guinea-pig aortic strip

(See Furchgott & Bhadrakom, 1953.) Contractions of the thoracic aorta were recorded either by an isotonic frontal writing lever (load 0·5 g; 10-fold magnification) on smoked paper or by a variable inductance transducer connected to a phase discriminator and pen recorder. Cumulative dose-response curves were obtained for noradrenaline, added to the organ bath every 5 minutes.

In experiments with angiotensin, submaximal doses were added for 3 min at intervals of 15 min, using three submaximal doses of noradrenaline in between to prevent tachyphylaxis to angiotensin.

Guinea-pig ileum

Longitudinal contractions of 2 cm lengths of ileum were recorded by isotonic frontal writing levers (1 g load; 5-fold magnification) on smoked paper. Acetylcholine (ACh) and histamine were added alternately for a contact time of 30 s in each 3 min period, with two changes of bath fluid between doses.

Guinea-pig trachea

The responses of the tracheal spiral (Constantine, 1965) to agonist drugs, which were added for 5 min in each 20–25 min period, were recorded by a variable inductance transducer connected to a phase discriminator and pen recorder.

Rabbit small intestine

(See Finkleman, 1930.) The spontaneous contractions of 3 cm lengths of intestine were recorded by isotonic frontal writing levers (load 1 g; magnification 7-fold) on smoked paper. At 3 or 4 min intervals the periarterial sympathetic nerves were stimulated (30–50 V, 0·1–1 ms, 6–20 Hz) for 20 s, or a concentration of noradrenaline ($1{\cdot}18 \times 10^{-7}$M), which caused almost complete inhibition of the spontaneous contractions was tested.

Rabbit heart

The Langendorff preparation of the rabbit heart was perfused retrogradely through a cannula in the aorta, using glass-filtered Krebs solution at a perfusion pressure of 40 cm H_2O. The force of spontaneous contraction of the left ventricle was recorded on smoked paper by a Starling heart lever and the heart rate was counted over 15 s periods.

Rat fundus

(See Vane, 1957.) Responses were recorded on smoked paper by an isotonic frontal writing lever (load 0·5 g; 16-fold magnification). Doses of ACh and 5-HT were added alternately for a contact time of 90 s in each 4 min period and washed out 3 times by overflow. The tissue was subjected to a stretching load of 0·5–1 g for 15 s after washing out each agonist dose.

Rat ileum

Longitudinal contractions of 2 cm lengths of terminal ileum were recorded with isotonic frontal writing levers (load 0·5 g; 7-fold magnification) on smoked paper. Doses of ACh and 5-HT were added alternately for a contact time of 30 s in each 4 min period and washed out three times by overflow.

Analysis of results

From experiments on the guinea-pig aortic strip and ileum and on the rat ileum, information was obtained on the nature of the antagonism of noradrenaline, histamine and 5-HT by indoramin. In each experiment, the mean dose-ratio was calculated from measurements (Gaddum, Hameed, Hathway & Stephens, 1955) taken at various corresponding points on the log dose-response lines obtained in the presence and absence of indoramin. This ratio was then subjected to analysis as described by Arunlakshana & Schild (1959). The pA_2 values were determined graphically and by calculation from $pA_2 = \log K_B = \log [(x-1)/B]$, where x=agonist dose-

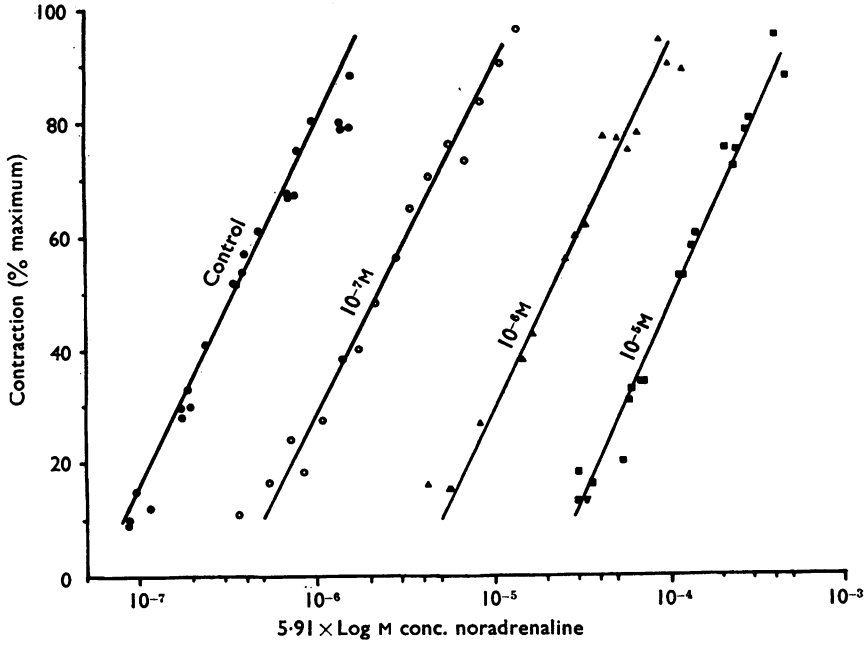

FIG. 1. Effect of indoramin hydrochloride on noradrenaline-induced contractions of the guinea-pig isolated aorta. Indoramin was added to the organ bath for 15 min before testing its effect. Each dose-response line has been obtained by superimposing the results of three to five experiments at the 50% maximal response level. Note the progressive parallel displacement of the line to the right of the control [control (n=5); 10^{-7}M (n=3); 10^{-6}M (n=4); 10^{-5}M (n=5)].

ratio, B=molar antagonist concentration and K_B=the affinity constant of the antagonist. pA_2 values on the aortic strip were also obtained directly (Schild, 1947). In addition the difference pA_2–pA_{10} was calculated ; for competitive antagonism this difference is 0·954 (Schild, 1947, 1957).

Drugs used

Acetylcholine bromide (B.D.H.), aminophylline (Burroughs Wellcome), (−)-ascorbic acid (B.D.H.), atropine sulphate (B.D.H.), dexamphetamine sulphate (Ward Blenkinsop), histamine acid phosphate (B.D.H.), (±)-propranolol hydrochloride (I.C.I.), indoramin hydrochloride (3-[2-(4-benzamidopiperid-1-yl)ethyl]indole hydrochloride, Wy 21901), (±)-isoprenaline sulphate (Burroughs Wellcome), (−)-noradrenaline bitartrate (Koch-Light), 5-hydroxytryptamine creatinine sulphate (Koch-Light), angiotensin amide (Ciba). All drug concentrations are expressed in terms of base.

Results

α-Adrenoceptor antagonism

Guinea-pig vas deferens

In concentrations of 10^{-7}M to 10^{-5}M, indoramin caused a dose related displacement of the log dose-response lines for noradrenaline to the right of the control. The

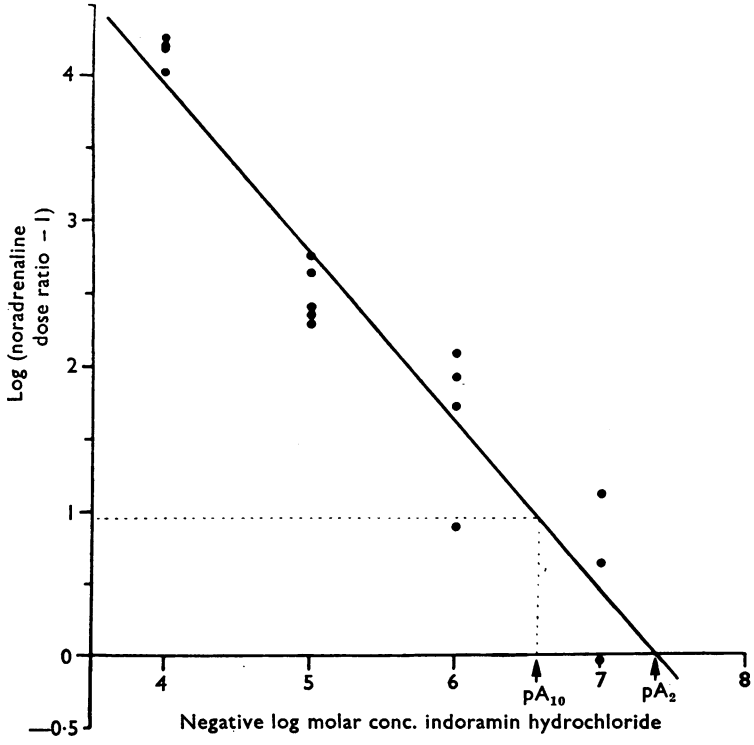

FIG. 2. Relation between the log (noradrenaline dose ratio−1) and the negative log of the molar concentration of indoramin hydrochloride, plotted by the method of Arunlakshana & Schild (1959). A calculated regression line is fitted to the sixteen points, each of which represents a mean dose-ratio for one experiment. Where log (dose-ratio−1) equals 0·954, the pA_{10} on the abscissa equals 6·57.

heights of the maximal responses were not reduced in the presence of indoramin. Equilibration of indoramin with the tissue occurred after 30 minutes. The antagonism produced by 10^{-5}M indoramin was not completely reversed after washing continuously by overflow for 2 h in antagonist-free Krebs solution.

Guinea-pig aortic strip

As with the vas deferens, the log dose-response lines for noradrenaline were displaced to the right of the control in a parallel dose-dependent manner by indoramin (10^{-7} to 10^{-4}M), with no effects on the heights of the maximal responses (Fig. 1). The solubility limit of noradrenaline in Krebs solution prevented maximal responses from being obtained in the presence of 10^{-4}M indoramin. Maximum antagonism was achieved by contact for 15 min with indoramin since no further displacement of the noradrenaline dose-response curve was obtained when the antagonist contact time was 30 min or 60 minutes. Reversal of the antagonism resulting from treatment with 10^{-5}M indoramin was complete after washing the tissue continuously by overflow for 3 h in antagonist-free Krebs solution.

The calculated mean dose ratios for noradrenaline increased with increasing concentrations of indoramin. The calculated regression line for the plot of log (nor-

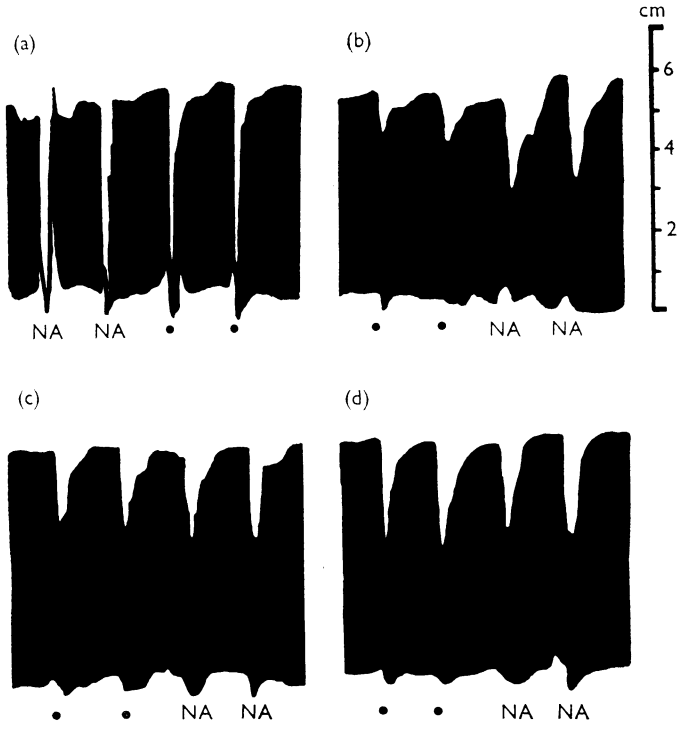

FIG. 3. Effect of indoramin on the inhibitory responses of the rabbit small intestine to stimulation of the periarterial sympathetic nerves and to added noradrenaline. At the black dots, the periarterial sympathetic nerves were stimulated at a frequency of 15 Hz with 0·1 ms duration at supramaximal voltage (30 V) for 20 seconds. At NA, noradrenaline ($1{\cdot}18\times10^{-7}$M) was added for a contact time of 15 seconds. The interval between successive periods of nerve stimulation or doses of noradrenaline was 4 minutes. The figure illustrates consecutive parts of an experiment: (a) initial responses, (b) responses after treatment with 10^{-5}M indoramin for 2 h, (c) responses after washing in antagonist-free Krebs solution for 30 min, (d) responses in the presence of $1{\cdot}85\times10^{-5}$M dexamphetamine.

adrenaline dose ratio−1) against negative log of molar concentration of indoramin had a slope of −1·16 and intersected the abscissa at the pA_2 value of 7·38 (Fig. 2). The calculated pA_2 values for each concentration of indoramin gave a mean of 7·48 (n=4). The direct pA_2 estimate was 7·41 (±0·12 S.E.; n=6). The experimental pA_2–pA_{10} difference was 0·81.

In experiments with noradrenaline and angiotensin, the responses to noradrenaline were blocked by 10^{-5}M indoramin whereas the angiotensin responses were unaffected.

Adrenergic neurone blockade

In the presence of 10^{-5}M indoramin, the spontaneous contractions of the rabbit intestine were initially reduced in size and often irregular, but after treatment for 1–2 h they had usually returned to near control levels and the responses to sympathetic nerve stimulation and noradrenaline could then be investigated. Both responses were reduced (Fig. 3a, b) and, on washing the tissue in antagonist-free Krebs solution, they remained reduced to the same extent (Fig. 3c). Treatment of the tissue with dexamphetamine ($3·70 \times 10^{-6}$ or $1·85 \times 10^{-5}$M) did not alter the inhibitory effect of periarterial nerve stimulation or noradrenaline (Fig. 3d).

In the absence of indoramin the responses to noradrenaline and sympathetic nerve stimulation were constant throughout an experiment of the same duration.

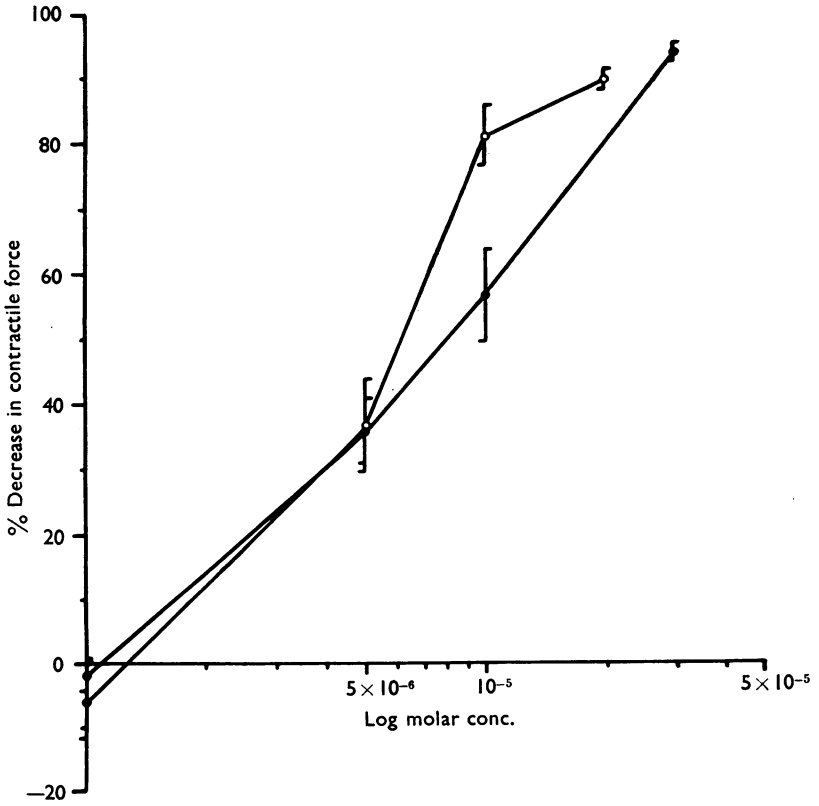

FIG. 4. Effect of indoramin (○) and propranolol (●) on the force of contraction of the rabbit isolated heart. Each point on the graph represents the mean and standard errors of three to five experiments.

β-Adrenoceptor antagonism

Guinea-pig trachea

Indoramin, even in a concentration of 10^{-4}M for 30 min, did not antagonize the relaxation induced by isoprenaline, noradrenaline or aminophylline.

Rabbit heart

Indoramin (10^{-6}M) perfused for 30 min had no significant effect on its own and did not alter the inotropic or chronotropic responses to aminophylline or isoprenaline. In contrast, propranolol (10^{-6}M) which also had no effect on its own, had no effect on the aminophylline responses but reduced the sensitivity to isoprenaline several hundred-fold.

Cardio-inhibitory properties

In concentrations above 10^{-6}M both indoramin and propranolol caused a similar and dose dependent reduction in the force (Fig. 4) and rate of contraction of the rabbit isolated heart. A partial reversal of the effects of both compounds occurred after perfusion with antagonist-free Krebs solution for 1 hour.

Antihistamine and anticholinergic activities

Guinea-pig trachea

Indoramin (10^{-6}M) for 30 min had no effect on ACh responses, but caused a

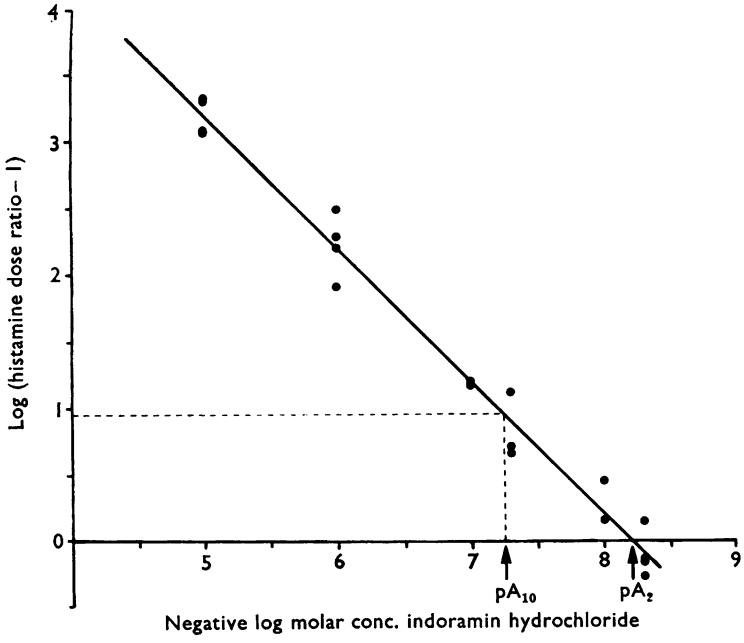

FIG. 5. Relation between the log (histamine dose ratio−1) and the negative logarithm of the molar concentration of indoramin hydrochloride, plotted by the method of Arunlakshana & Schild (1959). A calculated regression line is fitted to the twenty points, each of which represents a mean dose ratio for one experiment. Where log (dose ratio−1) equals 0·954, the pA_{10} on the abscissa equals 7·250.

parallel displacement of the histamine log dose-response lines to the right, without reducing the height of the maximal response.

Guinea-pig ileum

Similar results were obtained with the ileum. With indoramin (5×10^{-9}M to 1×10^{-5}M), the ACh responses were unaffected whereas the histamine log dose-response lines were displaced in a parallel manner to the right of the control without a reduction in the height of the maximal responses. Equilibration of indoramin with the tissue occurred after 15 minutes. The antagonism of histamine produced by 10^{-7}M or less of indoramin was reversed by washing within 25 min ; the reversal took longer for higher concentrations.

The calculated mean dose-ratios for ACh were all near unity over a 2,000-fold range of indoramin (5×10^{-9}M to 1×10^{-5}M) but, in contrast, the dose-ratios for histamine were progressively increased so that an approximately 1,600-fold increase in histamine dose was required to regain the control responses. The calculated regression line for the plot of log (histamine dose ratio -1) against negative log of molar concentration of indoramin had a slope of $-0{\cdot}99$ and intersected the abscissa at the pA_2 value of 8·205 (Fig. 5). The mean of the calculated pA_2 values for each concentration of indoramin was 8·229 and the experimental pA_2–pA_{10} difference was 0·955.

Antagonism of 5-hydroxytryptamine receptors

Rat fundus

Indoramin (10^{-5}M) for 10 min antagonized the action of 5-HT without affecting that of ACh.

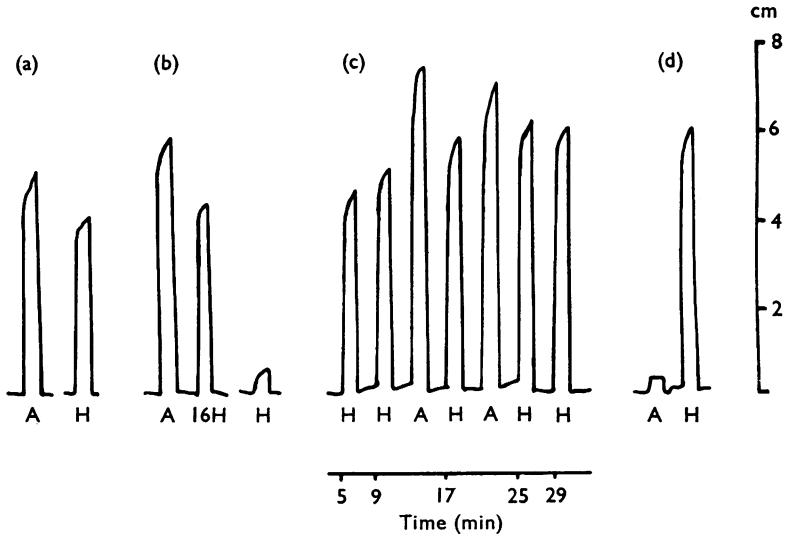

FIG. 6. Responses of the rat isolated ileum to doses of acetylcholine (A), $1{\cdot}37 \times 10^{-7}$M, and 5-hydroxytryptamine (H), $2{\cdot}27 \times 10^{-6}$M ; 16 H represents a dose of $3{\cdot}63 \times 10^{-5}$M. The figure illustrates consecutive parts of an experiment: (a) initial responses, (b) responses after treatment for 60 min with indoramin hydrochloride (10^{-5}M), (c) recovery of responses after washing for 5 min in antagonist-free Krebs solution, (d) responses after atropine ($1{\cdot}73 \times 10^{-8}$M) had been added to the Krebs solution for 5 minutes.

Rat ileum

Indoramin (10^{-6}M to 10^{-5}M) reduced the responses to 5-HT but not those to ACh (Fig. 6a, b). Equilibration of indoramin with the tissue occurred after 15 min and the heights of the maximal responses to 5-HT were not reduced. The antagonism was completely reversed within 25 min by washing in antagonist-free Krebs solution (Fig. 6c). Concentrations of indoramin above 10^{-5}M reduced both the ACh and 5-HT responses. Treatment with atropine almost completely blocked the response to ACh leaving that to 5-HT unaffected (Fig. 6d).

The calculated regression line for the plot of log (5-HT dose ratio -1) against negative log of molar concentration of indoramin had a slope of $-1{\cdot}13$ and intersected the abscissa at the pA_2 value of 5·98. The mean of the calculated pA_2 values for each concentration of indoramin was 6·05 and the experimental pA_2–pA_{10} difference was 0·80.

Discussion

The autonomic blocking properties of indoramin on isolated organs provide additional evidence for the possible mechanism of the hypotension produced in intact animals (Alps *et al.*, 1970c ; Alps *et al.*, 1970a). A dose-related peripheral vasodilator action of indoramin in the anaesthetized cat was ascribed to blockade of α-adrenoceptors because the vasoconstrictor responses to lumbar sympathetic nerve stimulation and injected noradrenaline were both reduced (Alps *et al.*, 1970c). The specificity of this α-adrenoceptor blockade was subsequently confirmed in the anaesthetized Red Patas monkey by the unaltered vasoconstrictor responses to 5-hydroxytryptamine and angiotensin (Alps, Johnson, Staniforth & Wilson, unpublished observations).

Results with the Finkleman preparation give further support to an α-adrenoceptor blocking effect of indoramin and exclude an adrenergic neurone blocking action, since the inhibitory responses to periarterial sympathetic nerve stimulation and to added noradrenaline were reduced to a similar extent and, unlike the antagonism produced by guanethidine (Day & Rand, 1963), dexamphetamine did not reverse the blockade. Experiments on the guinea-pig isolated aorta and vas deferens showed that indoramin exerted an α-adrenoceptor blocking action which, although of long duration, fulfilled the qualitative and quantitative criteria for competitive antagonism proposed by Gaddum (1957), Schild (1957), Furchgott (1955) and Arunlakshana & Schild (1959). Although quantitative comparisons with previously published work may be imprecise because of differences in experimental technique, the potency of the α-adrenoceptor blocking action of indoramin (pA_2 7·4) compared favourably with that of other α-adrenoceptor blocking drugs on vascular smooth muscle. On the rabbit aorta phentolamine's pA_2 was 7·1 (Urquilla, Stitzel & Fleming, 1970) ; that for piperoxan was 6·4 (Birmingham & Szolcsányi, 1965). Thymoxamine's pA_2 depended on the vascular preparation used and varied between 6·1 and 7·5 (Birmingham & Szolcsányi, 1965 ; Birmingham, Ernest & Newcombe, 1969). The pA_2 value of tolazoline was 5·5 (Furchgott, 1955) and that of dihydroergotamine varied from 7·7 to 8·3 (Furchgott, 1955).

Indoramin also had a specific and potent antihistamine action (pA_2 8·2) which again fulfilled all the qualitative and quantitative criteria for competitive antagonism but, unlike the α-adrenoceptor blockade, it was readily reversed on washing in

indoramin-free Krebs solution. In contrast to most other potent antihistamines, indoramin was without anticholinergic activity on the guinea-pig isolated ileum ($pA_2 < 5{\cdot}0$). On the rat isolated fundus and ileum indoramin was a weak antagonist of 5-HT; although the properties of the blockade fulfilled several of the criteria for competitive antagonism, the pA_2 value on the rat ileum was only 6·0. Neither the potent competitive antihistamine nor the weak 5-HT-antagonist activities are thought to be concerned in the hypotensive action of indoramin.

The possibility that the hypotensive action of indoramin is related to a direct relaxant action on the smooth muscle of the blood vessels is excluded by the specificity of the indoramin blockade of the noradrenaline responses of the aorta. This conclusion is also supported by the absence of a direct spasmolytic action of indoramin on any of the smooth muscle preparations which have been studied.

In the anaesthetized cat, the hypotensive action of indoramin is associated with a reduction in the rate and force of contraction of the heart (Alps *et al.*, 1970c). Although this cardio-inhibitory effect is similar to that produced by propranolol, it is not caused by β-adrenoceptor blockade since hypotensive and cardio-inhibitory doses of indoramin failed to block the responses of the heart to isoprenaline or cardiac nerve stimulation (Alps *et al.*, 1970a). These results on the rabbit isolated heart confirm the similar cardio-inhibitory actions of indoramin and propranolol. They also exclude β-adrenoceptor antagonism by indoramin because, unlike propranolol, indoramin failed to antagonize the responses to isoprenaline on both guinea-pig isolated trachea and rabbit isolated heart. It is tempting to speculate that the cardio-inhibition caused by indoramin may be related to its local anaesthetic membrane stabilizing action (Alps *et al,* 1970b) but, in view of evidence for the presence of α-adrenoceptors in the sinoatrial node (James, Bear, Lang & Green, 1968; Garvey, 1969) the α-adrenoceptor blocking action of indoramin may also influence its cardiac effects.

β-Adrenoceptor blocking drugs have been used clinically in the treatment of hypertension because of their cardio-inhibitory actions. Propranolol reduces cardiac output and lowers blood pressure in hypertensive patients (Prichard & Gillam, 1969), but the onset of the blood pressure fall is delayed and associated with an increase in vasoconstrictor tone (Frohlich, Tarazi, Dustan & Page, 1968). Alps, *et al.* (1970c) suggested that the disadvantage of the reflex vasoconstrictor response to β-adrenoceptor blocking drugs could be overcome by a combination of cardio-inhibition and peripheral α-adrenoceptor antagonism. We have shown that indoramin combines both of these properties and, unlike propranolol, its hypotensive action in intact animals is sustained and accompanied by an increase in peripheral blood flow (Alps *et al.*, 1970c).

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