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The α-adrenoceptor blocking effect of indoramin on human isolated smooth muscle

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The pharmacological effects of indoramin, a new hypotensive drug, were studied on human isolated smooth muscle preparations. Indoramin was shown to have a potent α-adrenoceptor blocking activity on specimens of colon, ileum and vein. The blockade fulfilled the established criteria for competitive antagonism of noradrenaline. The mean pA_2 values were 6·57 for the circular muscle of the colon, 6·91 for longitudinal muscle of the colon, 6·64 for longitudinal muscle of the ileum and 6·58 for saphenous vein. Indoramin (10^{-3}M) did not reduce acetylcholine-induced contractions of the longitudinal muscle of the colon and ileum.

Indoramin hydrochloride [3- (2-(4-benzamidopiperid-1-yl) ethyl) indole hydrochloride, Wy 21901], is a potent hypotensive agent, which in animals combines competitive α-adrenoceptor blocking and cardioinhibitory properties. It also has local anaesthetic, antihistamine and anti-5-hydroxytryptamine activity (Alps, Hill & others, 1970). The actions of indoramin on isolated preparations of human colon, ileum and saphenous vein have now been studied.

MATERIALS AND METHODS

Fresh specimens of human tissue from surgical operations were placed in Krebs bicarbonate solution at 5°, gassed with 5% carbon dioxide in oxygen, immediately after excision. Specimens of colon were obtained from tissue resected from patients with Crohn's disease, ulcerative colitis, diverticulitis or malignant tumours. The intestinal muscle was stripped of mucosa and mesentery and sections 2 × 40 mm long were cut as far as possible from any visible pathological lesions. The strips were cut in the direction of either the longitudinal muscle layer (taenia coli) or the circular muscle layer. The longitunal muscle layer was, however, preferred to the circular layer as it was more responsive to α-adrenoceptor agonist drugs (Coupar & Turner 1969; Hedges & Turner, 1969).

The specimens of ileum were all 'normal', resected from children at operations for ileocutaneous ureterostomy and ileal loop diversion. Specimens of saphenous vein were obtained from patients at operations for ligation and stripping of varicose veins. Spirals were cut after overlying fat and connective tissue had been removed.

Recording

The strips (approximately 3–5 cm long) were mounted in a 10–15 ml bath containing Krebs bicarbonate solution containing (g): NaCl 6·92, KCl 0·35, $MgSO_4$. $7H_2O$ 0·21, $CaCl_2.2H_2O$ 0·37; KH_2PO_4 0·16, $NaHCO_3$ 2·1 and glucose 2: distilled water to 1 litre.

The solution was gassed with 5% carbon dioxide in oxygen and the temperature maintained at 37 ± 0·25°. Isotonic responses of the smooth muscle strips were recorded on smoked paper with a frontal writing lever arranged to apply a tension of 0·5g for unstripped saphenous vein and 1g for a strip of colon or ileum at a lever magnification of approximately ×10. The smooth muscle preparations were left to equilibrate for at least 1 h before the addition of drugs.

Drugs

Noradrenaline (Levophed, Bayer Products Co). Indoramin (John Wyeth and Brother Ltd). Methacholine (Amechol, MacCarthy's Laboratories). Propranolol (ICI Ltd). Acetylcholine (Sigma Chemical Company).

pA_2 Determinations

The potency of indoramin was assessed by measuring the pA_2 value by the method of Arunlakshana & Schild (1959). The strips of colon or ileum were contracted by the addition of methacholine (250–500 ng ml^{-1}) to the Krebs solution, the amount of methacholine depended on individual strips. Normally, 250ng ml^{-1} methacholine was sufficient for strips of ileum obtained from young children. Propranolol (0·5 mg ml^{-1}) was added to the Krebs to block the β-adrenoceptors, thereby making it unlikely that the effect of indoramin on the tissue involved an action on these. Where the tissue recovery was rapid, most of the experiments were performed by the sequential dose method in which the dose of noradrenaline was added for a fixed contact time and then washed out with Krebs containing propranolol and methacholine, then the next higher dose of noradrenaline was added and this sequence was repeated until a maximal response to noradrenaline was obtained. Where recovery was slow, cumulative relaxations were produced by adding concentrations of (–)-noradrenaline to the bath at regular intervals, so that the maximum response occurred at each concentration. After obtaining constant control dose-response curves to noradrenaline a suitable dose of the antagonist indoramin ranging from $1·0 \times 10^{-7}$ to $3·16 \times 10^{-5}$M was placed in the reservoir containing the Krebs solution and the tissue allowed to equilibrate with it for 30 min before the addition of agonist; dose response curves at each level of antagonist concentration were thus obtained. An attempt was made at the end of the experiment to obtain responses similar to the agonist responses at the start of the experiment by repeated changing of the bath fluid to wash out the indoramin. In some cases an adjacent strip was set up under similar conditions and control responses obtained throughout the course of the experiment. If these did not alter significantly it was assumed that the changes measured in the test tissue were due entirely to the effect of the antagonist. The dose ratios of the agonist noradrenaline required to produce equivalent responses in three different concentrations of the antagonist, indoramin, were used to calculate the equilibrium pA_2 value of indoramin against noradrenaline.

Equilibrium pA_2 on human saphenous vein

Three strips of spirally cut human saphenous vein were used to test the effect of indoramin on the contractions produced by noradrenaline. Propranolol was not added, as the preparation contains mostly α-adrenoceptors with relatively few β-adrenoceptors (Coupar, 1970). Because of the time required (30–60 min) by the vein to

recover after each dose of agonist, it was necessary to make all the experiments by the cumulative dose response curve method. Cumulative contractions were produced by adding concentrations of noradrenaline to the bath at regular intervals until the maximum was reached. Subsequently, a dose-response curve (cumulative) at each level of indoramin was obtained. Mean dose ratios for noradrenaline in the presence of each of three concentrations of indoramin for each specimen of tissue were calculated and analysed by the method of Arunlakshana & Schild (1959). In most cases, an adjacent strip of vein was used as a control.

Effect of indoramin on acetylcholine

Three specimens of longitudinal muscle from colon and three specimens of longitudinal muscle from ileum were examined. Two constant dose response curves were first obtained to acetylcholine using a 5 min cycle, and a drug contact time of 30 s. A control response of approximately 50% maximal was then obtained. After obtaining several constant control responses to the agonist, a dose of indoramin was added and incubated with the tissue for 15 min, after which the control dose of acetylcholine was added and the response recorded.

RESULTS

The results of the pA_2 determinations on the various types of human tissue used are shown in Table 1.

Table 1. *Summary of the pA_2 values, the slope $-n$ of the log (dose ratio—1) versus log M (indoramin) graph and the pA_2 -pA_{10} values of indoramin against noradrenaline on various tissues.*

Tissue	No. of Determinations	pA_2 (mean & range)	Slope-n (mean & range)	pA_2-pA_{10} (mean & range)	Range of molar concentration of indoramin
Longitudinal colon	2	6·91 (6·80–7·01)	1·25 (1·0–1·5)	1·04 (0·78–1·3)	$1·18 \times 10^{-7}$ to $4·09 \times 10^{-6}$
Circular colon	1	6·57	0·8	1·1	$1·0 \times 10^{-7}$ to $3·16 \times 10^{-5}$
Longitudinal ileum	3	6·64 (6·27–6·87)	1·04 (0·8–1·33)	1·058 (0·99–1.24)	$1·18 \times 10^{-7}$ to $2·36 \times 10^{-4}$
Saphenous vein	3	6·58 (6·46–6·68)	0·79 (0·66–1·0)	1·45 (1·0–1·98)	$1·18 \times 10^{-7}$ to $1·25 \times 10^{-5}$

The average pA_2 values for indoramin show it to be about equipotent on all the tissues tested.

The mean pA_2 values of indoramin against noradrenaline were 6·91 for longitudinal colonic muscle, 6·57 for circular colonic muscle, 6·64 for longitudinal muscle of the ileum and 6·58 for saphenous vein.

In all the experiments indoramin caused a parallel displacement of the noradrenaline dose response curves to the right. Moreover, the heights of the maximal responses were not suppressed.

Effect of indoramin on acetylcholine responses. Indoramin up to a concentration of $4·4 \times 10^{-3}$M did not reduce the acetylcholine responses.

DISCUSSION

On human isolated smooth muscle preparations, indoramin has been found to possess potent α-adrenoceptor blocking activity, with pA_2 values of 6·27–7·01. This is in reasonably close agreement with the pA_2 value obtained from animal studies: for example, the pA_2 values obtained from the guinea-pig vas deferens and aortic strip were 7·4 (Alps, Hill & others, 1972). It is possible that changes in tissue responsiveness produced by premedication and anaesthesia of patients, together with species differences, may contribute to the observed difference in pA_2 values. In both man and animal studies, indoramin has been found to satisfy the established criteria for competitive antagonism (Arunlakshana & Schild, 1959). Briefly, in both investigations, indoramin caused a parallel dose-related shift of the log dose response curves for noradrenaline to the right of the control. The antagonism, although persistent, was surmountable. The slope of the plot log (dose-ratio −1) versus—log M concentration of indoramin was approximately equal to −0·97 (human study) and −1·16 (animal study). Moreover, the $pA_2 - pA_{10}$ values in the human study were in the range 0·78–1·98 while in the animal study it was found to be 0·81.

Indoramin up to a molar concentration of 10^{-3} (human tissue) and 10^{-5} (animal study) has been found to be devoid of any action on acetylcholine responses.

Some of these pharmacological actions of indoramin have been confirmed by clinical investigations. The competitive α-adrenoceptor blocking properties of indoramin were demonstrated in man by the parallel time-related shift to the right of the dose response curves to serial bolus doses of intravenous noradrenaline, after the intravenous administration of 20 mg of indoramin (Royds, Coltart & Lockhart, 1972). Clinical pharmacological studies also showed a reduction of phenylephrine-induced mydriasis, followed by a dose related miosis (Coltart, Lockhart & others, 1971). Further clinical evidence for α-adrenoceptor blockade was provided by a rise in the forearm blood flow and skin temperature of the hand after intravenous injection of indoramin (Royds & Lockhart, 1972). This α-adrenoceptor blocking action of indoramin probably contributes to the sustained nature of its hypotensive action which has been demonstrated in hypertensive patients (Royds, 1972).

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