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The effect of isoprenaline on the contraction of smooth muscle produced by histamine, acetylcholine or other agents

J. B. FARMER AND the late D. N. LEHRER

Isoprenaline given intravenously is a more potent antagonist of bronchospasm produced by histamine than of that produced by acetylcholine in the anaesthetised guinea-pig. This greater activity of isoprenaline against histamine was also observed on isolated tracheal muscle and ileum of the guinea-pig. It was also found in isolated guinea-pig ileum that stimulation of the periarterial sympathetic nerves produced a greater inhibition of contractions produced by histamine or 5-hydroxytryptamine than of those produced by acetylcholine or bradykinin. The inhibitory actions of isoprenaline were absent in tissues without β-receptors and tissues in which the β-receptors were blocked by pronethalol.

ISOPRENALINE is widely used, either alone or with other substances, as a bronchodilator. The bronchodilator action was first demonstrated in anaesthetised dogs and guinea-pigs (Konzett, 1940a, b) and has since been confirmed by many others. We have examined the inhibitory effect of isoprenaline on bronchoconstriction induced by a number of spasmogens in the anaesthetised guinea-pig and on the contractions of isolated smooth muscle preparations evoked by the same agents. Some of the results were communicated to the British Pharmacological Society in July 1965.

Experimental

In vivo EXPERIMENTS

The resistance of the guinea-pig lung to inflation was determined by the method of Konzett & Rössler (1940). Guinea-pigs, 250–350 g, were anaesthetised with urethane, 175 mg/100 g, by intraperitoneal injection. Spontaneous respiratory movements were suppressed by the additional intravenous injection of pentobarbitone. The trachea was cannulated and the lungs inflated with air by a miniature Starling pump operating on a partly closed circuit (4–6 ml stroke volume at 72 strokes/min).

Solutions of drugs in saline were injected through a cannula placed in the jugular vein. Temporary increases in bronchial resistance were produced by the intravenous injection of histamine and acetylcholine.

In vitro EXPERIMENTS

Guinea-pig ileum. A segment of ileum 2–3 cm in length was removed 20 cm from the ileocaecal junction. The segment was suspended in a 25 ml bath containing a physiological salt solution at 32° and the tissue aerated by means of a Hiflo pump. The composition of the salt solution in g/litre was $NaHCO_3$, 1·0; NaH_2PO_4, 0·32; NaCl, 8·0; glucose, 1·0; $MgCl_2$, 0·42; KCl, 0·2; $CaCl_2$, 0·4.

Mechanical records were obtained by means of an isotonic lever with a frontal writing point. The lever exerted a tension of 1 g and magnified

From the Department of Pharmacology, Allen & Hanburys Ltd., Ware, Herts.

the contractions 7 times. pA_2 determinations at 2 min were made by the method of Schild (1947). In the experiments in which chlorpheniramine and atropine were used, these drugs were incorporated in the salt solution for the duration of the experiment.

The periarterial nerves were stimulated as has been previously described for the rabbit ileum (Finkelman, 1930) The preparation was stimulated at variable frequencies with rectangular pulses of 1 msec duration for periods of 10 sec before and during the administration of the spasmogen. The strength of the shocks was such that the responses for a given frequency of stimulation were maximal.

Guinea-pig tracheal chain. The tracheal chain preparation was set up as described by Castillo & de Beer (1947) except that the tracheal rings were opened by severing the cartilage (Akcasu, 1959). The tracheal chain was suspended in a salt solution (composition as above) in a manner similar to that described for the ileum.

Human myometrium. Segments of non-gravid human uteri were prepared as described by Chambers & Pickles (1958) and suspended in 50 ml of aerated salt solution at 37°. The salt solution had the following composition, g/litre: NaCl, 9·0; KCl, 0·42; $CaCl_2$, 0·24; $NaHCO_3$, 0·5; glucose, 1·0. Contractions of the muscle were recorded with an isotonic frontal writing lever which exerted a tension of approximately 1 g and magnified the contractions 10 times.

DRUGS

Histamine acid phosphate; acetylcholine chloride; pronethalol hydrochloride; papaverine hydrochloride; isoprenaline sulphate; chlorpheniramine maleate; atropine sulphate; 5-hydroxytryptamine creatinine sulphate; bradykinin (Sandoz). Doses are given in terms of these salts.

Results

EFFECT OF ISOPRENALINE ON THE BRONCHOCONSTRICTOR ACTION OF HISTAMINE AND ACETYLCHOLINE IN THE GUINEA-PIG

Fig. 1 shows the inhibitory effect of intravenously injected isoprenaline on the bronchoconstrictor action of histamine and acetylcholine in the anaesthetised guinea-pig. Responses to histamine and acetylcholine were determined before, and at 20 min intervals after injection of isoprenaline (Fig. 1). The response produced by acetylcholine returned to near normal within 200 min, whereas the response to histamine was still reduced. Much smaller doses of isoprenaline were also effective but the duration of their effects were relatively brief. The results obtained with such doses against histamine and acetylcholine are expressed graphically in Fig. 2. The degree of inhibition of the bronchoconstriction induced by histamine was 30% and 90% at doses of 0·1 and 1·0 μg/kg of isoprenaline. The doses of isoprenaline required to reduce the bronchoconstriction induced by acetylcholine to a similar degree were 1·0 and 10·0 μg/kg respectively. Thus some ten times the amount of isoprenaline is

required to antagonise bronchoconstriction induced by acetylcholine than to antagonise that produced by histamine.

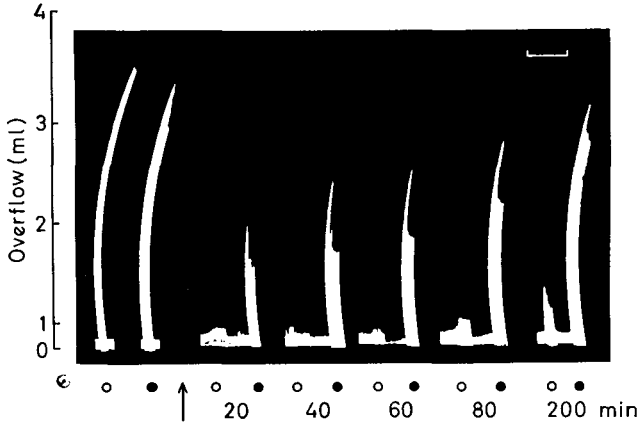

FIG. 1. Guinea-pig, 350 g. The effect of isoprenaline sulphate on the broncho-constrictor action of histamine and acetylcholine. Histamine (○, 2·5 μg) and acetylcholine (●, 6 μg) were injected intravenously. 20 min elapsed between each injection of the same drug, acetylcholine being injected 5 min after each injection of histamine. At ↑, isoprenaline sulphate, 1 mg/kg, was injected.

MODIFICATION OF THE EFFECT OF ISOPRENALINE BY PRONETHALOL

The inhibitory action of isoprenaline against bronchospasm induced in the anaesthetised guinea-pig by histamine and acetylcholine was antagonised by the prior administration of pronethalol. The effects of isoprenaline were investigated against histamine and acetylcholine in separate experiments since much smaller doses of isoprenaline were required to antagonise the histamine induced bronchospasm. The doses of isoprenaline employed against histamine and acetylcholine were 10 and 100 μg/kg respectively, and these regularly caused complete antagonism of the histamine and acetylcholine responses. In any one experiment, constant responses to injections of histamine or acetylcholine were obtained, then an intramuscular injection of pronethalol (0·1 mg/kg) was given. Pronethalol enhanced bronchoconstrictor actions of histamine and, to a lesser extent, of acetylcholine. When the responses were constant, an intravenous injection of isoprenaline was given. After the effects of isoprenaline had subsided, a second dose of pronethalol was given (1 mg/kg) and this was followed by another dose of isoprenaline. This sequence was repeated a third time, after injection of 10 mg/kg pronethalol. The results obtained are shown in Fig. 3. The responses to histamine (open columns) and acetylcholine (closed columns) obtained 3 min after the injection of isoprenaline are expressed as a percentage of the response to histamine or acetylcholine after the injection of pronethalol but before the isoprenaline administration. In the figure, A refers to the

depressed responses produced by isoprenaline in the absence of pronethalol; B, C and D refer to the responses obtained after pronethalol (0·1, 1·0 and 10·0 mg/kg respectively). Doses of isoprenaline which were equi-effective against histamine and acetylcholine induced bronchospasm were antagonised to a similar degree by pronethalol.

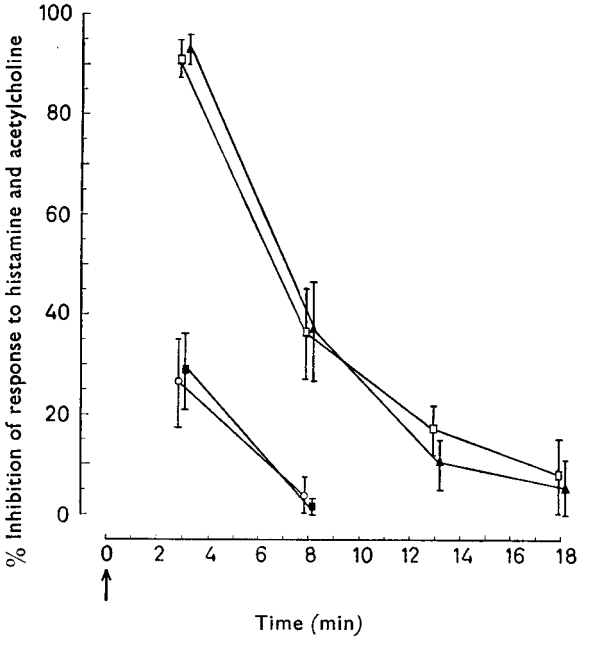

FIG. 2. The effect of graded doses of isoprenaline sulphate, 0·1 (○), 1·0 (□) and 10·0 (△) μg/kg intravenously at ↑ on the bronchoconstrictor actions of histamine (open symbols) and acetylcholine (closed symbols), in the anaesthetised guinea-pig. The points shown are mean result ± standard error for a group of 4 animals.

EFFECT OF ISOPRENALINE ON THE CONTRACTILE RESPONSES OF ISOLATED TISSUES TO HISTAMINE AND ACETYLCHOLINE

Tracheal chain preparation of the guinea-pig. The effects of isoprenaline on the contractile responses of tracheal chains to submaximal doses of histamine and acetylcholine were examined in four preparations. In all experiments, isoprenaline antagonised the response to histamine to a greater extent than it antagonised that to acetylcholine. For example it was found that concentrations of 0·5 to 1·0 ng/ml isoprenaline produced a marked reduction in the response of the tracheal chain to histamine with little or no effect on the response to acetylcholine. However 2·0 ng/ml isoprenaline was found to reduce the responses to both acetylcholine and histamine.

Guinea-pig ileum. The pA_2 value at 2 min was determined for isoprenaline against histamine and acetylcholine. These values are given as the mean value with the number of determinations in brackets. The values

found were 7·73 ± 0·11 (4) against histamine and 6·89 ± 0·05 (3) against acetylcholine. The difference in the pA_2 values was highly significant ($P < 0.001$). These pA_2 values for isoprenaline were found to be raised by the addition of atropine or chlorpheniramine to the bathing fluid.

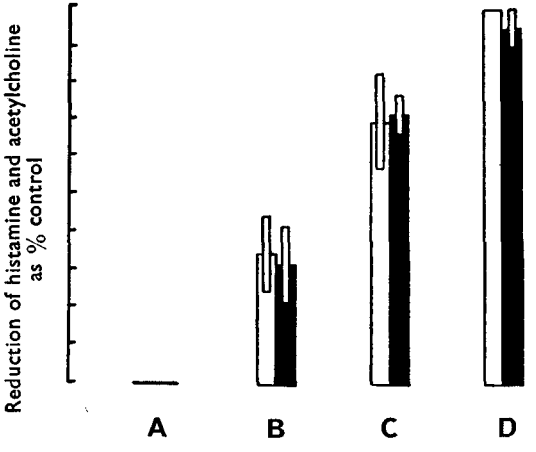

FIG. 3. The inhibitory action of isoprenaline, 10 μg/kg, on the bronchoconstrictor action of histamine (open columns) and of isoprenaline, 100 μg/kg, on the bronchoconstrictor action of acetylcholine (closed columns). Ordinate: height of response to histamine or acetylcholine 3 min after injection of isoprenaline expressed as a percentage of control (see text). A, B, C and D are mean results ± standard error for groups of 4 animals which had received prior injection of saline (A) or 0·1 (B), 1·0 (C) and 10 (D) mg/kg pronethalol intramuscularly.

Thus atropine 40 ng/ml, did not affect the response of the ileum to histamine but increased the pA_2 value for isoprenaline against histamine from 7·73 ± 0·11 (4) to 10·18 ± 0·36 (8). On the other hand, chlorpheniramine at 40 ng/ml which possessed no anti-acetylcholine action, increased the pA_2 value for isoprenaline against acetylcholine from 6·89 ± 0·05 (3) to 8·08 ± 0·19 (3). The concentrations of atropine and chlorpheniramine employed abolished the responses of the ileum to acetylcholine and histamine respectively.

The inhibitory effect of stimulation of sympathetic periarterial nerves on the response of the ileum to acetylcholine, histamine, 5-hydroxytryptamine (5-HT) and bradykinin was also examined. It did not prove practicable to examine such inhibitory effects against these four substances on any one piece of tissue, so the effect of periarterial nerve stimulation on the responses of the guinea-pig ileum to histamine, 5-HT and bradykinin was individually compared with the effect against acetylcholine in each experiment. Three experiments were made for each investigation and examples of the results are shown in Fig. 4. In Fig. 4 the effect of nerve stimulation on the responses of the ileum to 5-HT and acetylcholine were compared. The response to 5-HT was abolished, but the response to acetylcholine although markedly reduced was not abolished. Even a

four-fold increase in the concentration of 5-HT did not produce a contraction during nerve stimulation. The response to histamine was more reduced than the response to acetylcholine (Fig. 4). However the responses to acetylcholine and bradykinin were equally depressed by sympathetic nerve stimulation (Fig. 4). Thus the order of potency for the inhibitory effect of sympathetic nerve stimulation against these substances is 5-hydroxytryptamine > histamine > acetylcholine = bradykinin.

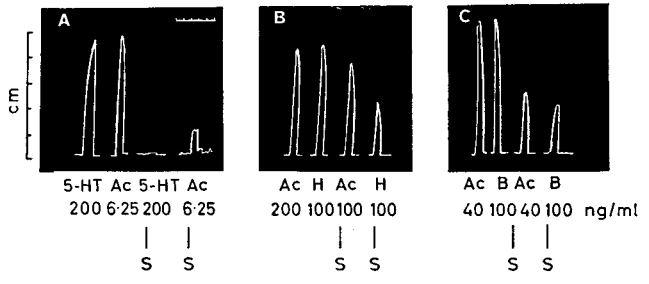

FIG. 4. The effect of periarterial sympathetic nerve stimulation (S) on the response of the guinea-pig ileum to acetylcholine (Ac), 5-hydroxytryptamine (5-HT), histamine (H) and bradykinin (B). Time scale: min.

Human myometrium. The effects of isoprenaline on spontaneous activity, and on the response of the human myometrium to histamine and acetylcholine were examined in three preparations. The results of one experiment are shown in Fig. 5. The isolated human myometrium contracted when histamine or acetylcholine was added to the bathing fluid. These responses were superimposed on the spontaneous contractions of the tissue. Concentrations of up to 40 μg/ml isoprenaline did not affect the responses to histamine or to acetylcholine, nor was there any reduction in the spontaneous motility of the tissue. Papaverine in a concentration of 10 μg/ml caused a relaxation of the tissue, decreased the spontaneous contractions and inhibited the contractions produced by histamine and acetylcholine.

Discussion

The results presented demonstrate the greater inhibitory action of isoprenaline against histamine than against acetylcholine-induced spasm of guinea-pig tissues. In unpublished experiments we have observed that this property is shared by adrenaline and noradrenaline, although on a quantitative basis these amines are less active. A similar qualitative picture was obtained for the inhibitory action of sympathetic nerve stimulation on the response of the guinea-pig ileum to histamine and acetylcholine. A related observation was made by Wilson (1964) who observed that phenylephrine, noradrenaline, adrenaline and isoprenaline were more active in antagonising the response of the guinea-pig ileum to histamine than to methacholine. Thus catecholamines show specificity in their inhibitory actions against responses to spasmogens.

Experiments were made to determine whether the inhibitory action of isoprenaline against spasmogens may be explained by its activation of β-adrenoceptive receptors. The β-blocking agent pronethalol was used to block the β-adrenoceptive receptors (Black & Stephenson, 1962).

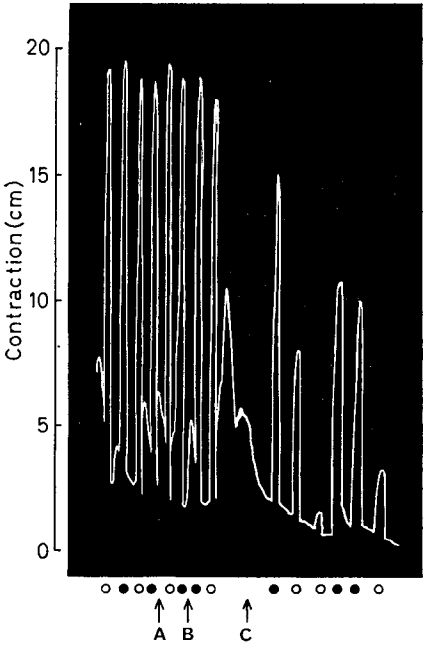

FIG. 5. Isolated human myometrium. The effect of isoprenaline 4·0 (A), 40·0 (B) μg/ml and papaverine 10 μg/ml (C) on spontaneous activity, and histamine (○) and acetylcholine (●)-induced contractions. Tissue suspended in Locke solution at 37°.

Doses of isoprenaline which produced equi-effective depressions of the bronchoconstrictor action of histamine and acetylcholine were antagonised to the same extent by equal doses of pronethalol, and it is therefore likely that only one type of β-receptor is involved. Since pronethalol completely antagonised the effect of isoprenaline on both histamine and acetylcholine, without itself suppressing the response to histamine and acetylcholine, it is unlikely that there is any direct interaction of isoprenaline with histamine and acetylcholine receptors. Secondly, evidence is available that the human myometrium contains few or no β-adrenergic receptors (Lehrer, 1965). This preparation readily contracted to histamine and acetylcholine but neither of these responses, nor the spontaneous contractions of the tissues, were reduced by isoprenaline. Papaverine (10 μg/ml) on the other hand caused relaxation of the tissue, decreased spontaneous contractions and reduced those due to histamine and acetylcholine. It was reasoned that should isoprenaline occupy histamine and to a lesser extent acetylcholine receptors, then the addition of an antihistamine might

enhance the activity of isoprenaline against acetylcholine. In fact the pA_2 value for isoprenaline versus acetylcholine was raised by the addition of chlorpheniramine to the bathing fluid, but the pA_2 value for isoprenaline versus histamine was increased to a greater extent by the presence of atropine. Clearly these results do not lend support to an action of isoprenaline on histamine or acetylcholine receptors, but they do suggest an interaction of isoprenaline with both atropine and chlorpheniramine at some other receptor sites.

Thus it would seem that the inhibitory actions of catecholamines injected, or released from sympathetic nerves show specificity in their actions against spasmogens. This is not due to any direct interaction of the catecholamines with the various receptors for these spasmogens but is a result of activation of adrenoceptic receptors.

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