J. Pharm. Pharmac., 1971, 23, 98–101 *Received July* 31, 1970

The action of isoprenaline on the perfused vessels of the rabbit ear

M. D. DAY AND HAZEL V. R. DIXON

Pharmacological Laboratories, Department of Pharmacy, University of Aston in Birmingham, Gosta Green, Birmingham 4, *U.K.*

In rabbit isolated perfused ear preparations isoprenaline in doses of 1 to 5 μg caused a vasodilatation which was specifically blocked by propranolol. In a minority of experiments larger doses of isoprenaline (5 to 10 μg) caused vasoconstriction. The vasodilatations to isoprenaline were increased by raising vascular tone with barium chloride whereas ergotamine and sympathetic stimulation were relatively ineffective. In the isolated ear central artery preparation, isoprenaline caused a vasoconstriction due to α-receptor stimulation. A vasodilator response due to β-receptor stimulation was revealed when perfusion pressure was raised with barium chloride. It is concluded that α- and β-adrenergic receptors are present in both preparations but a higher proportion of β-receptors are present in the whole ear.

The isolated perfused artery preparation from the rabbit ear was first described by de la Lande & Rand (1965) and has since been extensively used for studying the effects of vaso-active drugs (for instance de la Lande & Harvey, 1965; Starr & West, 1966; Day & Owen, 1968).

Gay, Rand & Wilson (1967) investigated the action of isoprenaline on this preparation and found that under a variety of conditions it produced only vasoconstrictor responses which were abolished by α-adrenergic receptor blocking agents. These workers concluded that isoprenaline was acting on α-adrenergic receptors in this preparation and that β-receptors were either absent or stimulation of them did not elicit vasodilator responses.

In view of the widespread use of this preparation for pharmacological studies it was thought worthwhile to re-examine it for the presence of β-adrenergic receptors.

METHODS

Whole ear preparation. A polyethylene cannula was used to cannulate the central artery and the vessels were perfused with Tyrode solution gassed with air and maintained at 37° by means of a constant volume flow inducer. The rate of perfusion varied between 5 and 8 ml/min in different preparations and perfusion pressure was recorded using a mercury manometer.

Isolated artery preparation. This tissue was set up as described by de la Lande & Rand (1965) except that aerated Tyrode solution at 37° was the perfusion fluid and the vessel was suspended in air, not in an organ bath containing the perfusion fluid.

In both preparations the Tyrode solution was filtered through resin filter paper immediately before use. Drugs were injected in volumes not exceeding 0·1 ml into the cannula via a short polyethylene tube fitted with a one way tap system. Each drug injection was given at a constant rate of 0·1 ml/15 s and was flushed in with a further 0·2 ml Tyrode solution injected at the same rate. Infusions were made by addition of the drug to the Tyrode solution in the reservoir.

Sympathetic stimulation was achieved by threading the vessel through a bipolar ring electrode or with the whole ear preparation by using a bipolar hook electrode placed under the upper end of the artery. Stimulation was at supramaximal voltage (10 to 25 V) and at a frequency of 2 to 10 pulses/s.

Drugs

Drugs used were: acetylcholine chloride, isoprenaline sulphate (±) and (−) -noradrenaline hydrochloride, propranolol hydrochloride (Inderal, ICI), phentolamine mesylate (Rogitine, Ciba), ergotamine tartrate (Femergin, Sandoz).

All doses are expressed in terms of the salt.

To avoid loss of potency, solutions of noradrenaline and isoprenaline were made up in 0·01N hydrochloric acid and kept on ice throughout the experiment. This vehicle was without effect on perfusion pressure when injected in volumes similar to those used for drug injections.

RESULTS

Whole ear preparation

In 9 of 13 preparations injections of isoprenaline (1 to 5 μg) caused vasodilatations with a fall in perfusion pressure of 5 to 25 mm Hg. In four of the preparations which responded to low doses of isoprenaline with vasodilations, higher doses caused biphasic responses with a marked vasoconstrictor component with increasing dosage. This is illustrated in Fig. 1A where 1 μg of isoprenaline caused predominantly vasodilatation whereas 10 μg caused a marked vasoconstriction. The vasodilator responses to isoprenaline were specifically abolished by propranolol (25 to 100 ng/ml). This is

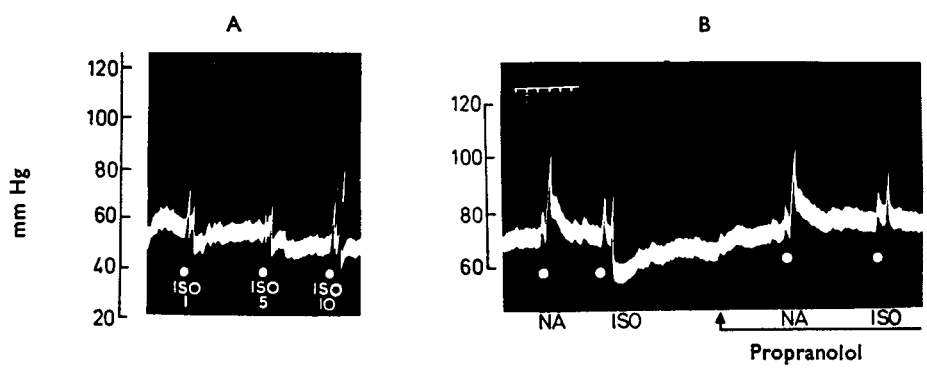

FIG. 1.A. Whole ear preparation: Response to isoprenaline (ISO) 1, 5 and 10 μg showing change from predominantly vasodilator response at 1 μg to predominantly vasoconstrictor response at 10 μg. B. Whole ear preparation: Responses to noradrenaline 50 ng (NA) and isoprenaline 1 μg (ISO) before and in the presence of propranolol 25 ng/ml. Time scale in min.

shown in Fig. 1B where the response to isoprenaline (1 μg) was converted from a dilatation to a constriction in the presence of propranolol whereas the constrictor response to noradrenaline was unaffected. The vasoconstrictions to both isoprenaline and noradrenaline were in all experiments abolished by phentolamine (1 μg/ml). In nine experiments of this series only vasodilatations could be obtained with any dose of isoprenaline in the absence of propranolol.

Effect of isoprenaline administered during raised perfusion pressure

Ergotamine. Ergotamine (2 to 10 ng/ml) raised the perfusion pressure from a resting level of 30 to 60 mm Hg to a level of 80 to 110 mm Hg. However, the increased perfusion pressure was not well maintained and in only two out of five preparations did isoprenaline cause a small fall in perfusion pressure of about 5 mm Hg. No constrictor action of isoprenaline (10 to 100 μg) was obtained in the presence of ergotamine.

Sympathetic stimulation. Arterial spasm was produced by sympathetic stimulation at frequencies of 2 to 10 pulses/s but in only three preparations out of 19 tested was the increased perfusion pressure sufficiently well maintained to enable isoprenaline to be injected during stimulation. In each of these three experiments isoprenaline caused an enhanced vasodilatation compared with the response before stimulation.

Barium chloride. Barium chloride (50 to 500 μg/ml) caused a sustained increase in perfusion pressure and in four out of five experiments the vasodilatation in response to isoprenaline was markedly increased. This is illustrated in Fig. 2 where the vasodilatations to both isoprenaline and acetylcholine are compared before and in the presence of barium chloride. The vasodilatation to isoprenaline was increased more markedly than was that to acetylcholine in the presence of barium ions.

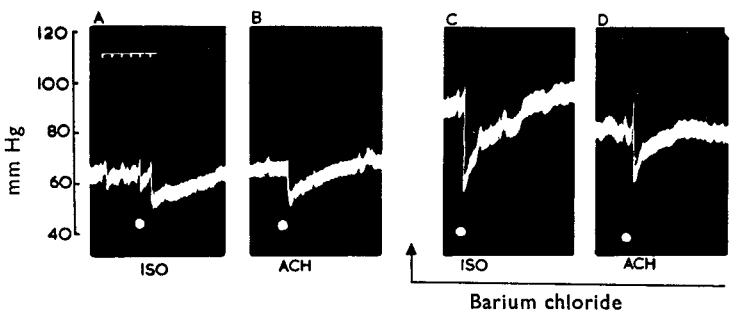

FIG. 2. Whole ear preparation: Responses to isoprenaline 1 μg (ISO) and acetylcholine 1 μg (ACH) before (panels A and B) and in the presence of barium chloride 100 μg/ml (panels C and D). Time scale in min.

The isolated artery preparation

In doses ranging from 1 to 10 μg isoprenaline caused either no change in perfusion pressure or else a small vasoconstriction. In larger doses (10 to 100 μg) it caused a marked vasoconstriction which was abolished by phentolamine (1 μg/ml) confirming the results of Gay & others (1967).

Barium chloride. In the presence of barium chloride (50 to 500 μg/ml) a sustained increase in perfusion pressure occurred and in four out of five preparations a marked vasodilatation occurred in response to injections of isoprenaline (5 to 10 μg). Fig. 3 illustrates an experiment in which vasodilatations in response to isoprenaline (5 and 10 μg doses) were revealed in the presence of barium chloride (100 μg/ml) and were abolished by propranolol (50 ng/ml).

Sympathetic stimulation. Vasodilatations in response to isoprenaline were not obtained in any preparation in which the perfusion pressure was raised by sympathetic stimulation (4 experiments).

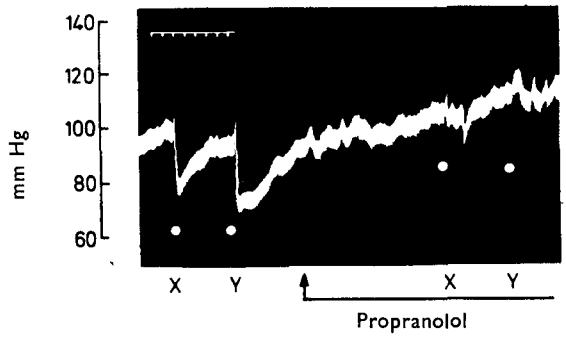

FIG. 3. Isolated central artery preparation: Perfusion pressure raised by the addition of barium chloride (100 μg/ml) present throughout the experiment. The vasodilatations in response to isoprenaline 5 μg (X) and 10 μg (Y) were abolished in the presence of propranolol (50 ng/ml). Time scale in min.

DISCUSSION

The results obtained in this study indicate that isoprenaline has both α and β-adrenergic receptor stimulant properties on the vasculature of the rabbit ear. It was easier to demonstrate a vasodilator response to isoprenaline in the whole ear preparation, even in the absence of raised vascular tone, than in the isolated artery preparation. In addition, it was more difficult to reveal an α-stimulant action of isoprenaline on the whole ear preparation than in the isolated artery. The most likely explanation of these observations is that the whole ear preparation contains a larger proportion of β-receptors, presumably present in the small arterial branches and in the veins, than are present in the arterial preparation. Raising the tone of the whole ear preparation with barium chloride markedly increased the dilatations in response to isoprenaline whereas sympathetic stimulation and ergotamine were relatively ineffective.

The present results confirm the observation of Gay & others (1967) that in the isolated artery preparation the main effect of isoprenaline is vasoconstriction due to α-adrenergic receptor stimulation. We also confirmed their observation that raising the tone by sympathetic nerve stimulation did not reveal a vasodilatation in response to isoprenaline. However, a β-receptor stimulant effect of isoprenaline was readily revealed when the perfusion pressure was raised by adding barium chloride to the perfusion solution. Thus it seems likely that both α- and β-adrenergic receptors are present in the central artery of the rabbit ear. The failure in the present experiments as well as in those of Gay & others (1967) to reveal a β-stimulant action of isoprenaline in the presence of ergotamine may indicate a β-receptor blocking action of this substance.

REFERENCES

DAY, M. D. & OWEN, D. A. A. (1968). *Br. J. Pharmac.*, **34**, 499–507.
DE LA LANDE, I. S. & HARVEY, J. (1965). *J. Pharm. Pharmac.*, **17**, 589–593.
DE LA LANDE, I. S. & RAND, M. J. (1965). *Aust. J. exp. Biol. med. Sci*, **43**, 639–656.
GAY, W. S., RAND, M. J. & WILSON, J. (1967). *J. Pharm. Pharmac.*, **19**, 468–473.
STARR, M. S. & WEST, G. B. (1966). *Ibid.*, **18**, 838–840.