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Factors influencing the adrenergic neurone blocking action of propranolol

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Summary

1. The reversal by propranolol of its own adrenergic neurone blocking effect in the cat can be prevented by cutting the splanchnic nerves or by ligating the adrenal veins.

2. In the absence of secretion from the adrenal medulla the nerve blocking action of propranolol is more complete, but can still be reversed by repeated injections or a constant infusion of adrenaline.

3. Prior treatment with adrenaline or noradrenaline also prevents the development of the blocking action of propranolol in the cat and in the isolated guinea-pig vas deferens.

4. It is suggested that in the cat, propranolol stimulates the release of catecholamines from the adrenal medulla which antagonize its nerve blocking effect.

Introduction

In an earlier publication it was shown that propranolol reduced the effect of adrenergic nerve stimulation in the cat, when given in small doses (25–100 μg/kg), without significantly altering the responses to injected noradrenaline (Eliash & Weinstock, 1971). Doses in excess of 100 μg/kg did not reduce the effect of nerve stimulation. Furthermore, nerve blockade, once established by the injection of several small amounts of propranolol could be reversed by giving increasing doses of the drug.

In 1966, Kayaalp & Kiran showed that propranolol (1 mg/kg) produced a sustained vasoconstriction in the perfused hind limb of the dog. This effect was blocked by phenoxybenzamine and by adrenal vein ligation. In the rat also, it was found that propranolol caused a prolonged rise in blood pressure (Dasgupta, 1968; Yamamoto & Sekiya, 1969; Regoli, 1970). The pressor response did not occur in the absence of the adrenal medulla (Regoli, 1970). These findings suggested that propranolol released catecholamines from the adrenal medulla which are responsible both for the localized vasoconstrictor effect in the dog and the pressor response in the rat.

It was possible that in the cat too, propranolol releases catecholamines from the adrenal medulla, and that these contribute to, or are responsible for, the reversal by propranolol of its own adrenergic neurone blocking action. We therefore decided to compare the effect of propranolol in normal and adrenalectomized cats, and also to study the effect of applied adrenaline on the blockade. The effect of

adrenaline was also determined on the nerve blockade produced by propranolol in the guinea-pig isolated vas deferens preparation.

Methods

Cats

Male and female cats weighing 2·0–3·5 kg were anaesthetized with ether and chloralose 75–80 mg/kg. In each of 10 animals both adrenal veins were tied and the glands were left *in situ*. The cats were given one injection of cortisone 50 mg/kg intramuscularly before the veins were tied, and then 0·25 mg/kg hydrocortisone solution, intravenously every hour throughout the experiment. In this way the blood pressure was maintained at a constant level. In four other cats both splanchnic nerves were cut close to the adrenal glands. Three hours were allowed to elapse before injections of propranolol were given.

The supe· ɔr cervical nerve was stimulated pre-ganglionically for 15 s at 10 Hz, 0·5 ms d· .ion every 2 minutes. Contractions of the nictitating membrane were recor· ' means of a Grass displacement transducer, FT03B on a multichannel po' Blood pressure was recorded with a Statham pressure transducer ' a cannula in the femoral artery. Infusions of adrenaline or nor- were made through a cannula in one femoral vein with a B. Braun a infusion pump. Drugs were injected into the second femoral vein.

Guinea-pig vas deferens

·e isolated guinea-pig vas deferens was set up as described by Huković (1961) .. a 50 ml gut bath. The hypogastric nerve was stimulated at 80 Hz, 2 ms duration for 5 s every minute. A voltage was used which gave contractions 90% of maximal. Recordings were made with a Grass displacement transducer FT03B on a multichannel recorder.

Propranolol was injected every 20 min and its effect on the contractions determined. In some experiments adrenaline was injected into the bath at a dose which by itself did not noticeably alter the effect of nerve stimulation. This dose was given every 5 min while the effect of propranolol was being studied. In other experiments, adrenaline was injected every 5 min after the blockade had already been established.

The drugs used were (±)-propranolol hydrochloride (Deralin, Abic Ltd.), (±)-adrenaline hydrochloride, (±)-noradrenaline bitartrate, cortisone acetate (suspension), hydrocortison· , alcoholic solution (Hydro-Adreson, Organon Ltd.). The drugs were dissolved in 0·9% w/v sodium chloride solution before injection. All doses are expressed in weight of the salt per kg body weight.

Results

Cat experiments

Doses of propranolol as low as 10 μg/kg produced significant nerve blockade in many of the cats. Additional amounts of propranolol of 100–200 μg/kg injected at 20 min intervals caused no further depression in the response of the nictitating membrane. Dose increments from 0·4 to 1·6 mg/kg produced a gradual restoration of the responses to nerve stimulation (see Fig. 1).

Propranolol also reduced the effects of nerve stimulation at doses of 10–100 μg/kg in cats with ligated adrenal veins. In this preparation, however, increases in the dose of the drug up to 1·6 mg/kg did not reverse the nerve blockade, but further diminished the responses until no contractions of the nictitating membrane were seen (see Fig. 1). Complete nerve blockade with no subsequent reversal

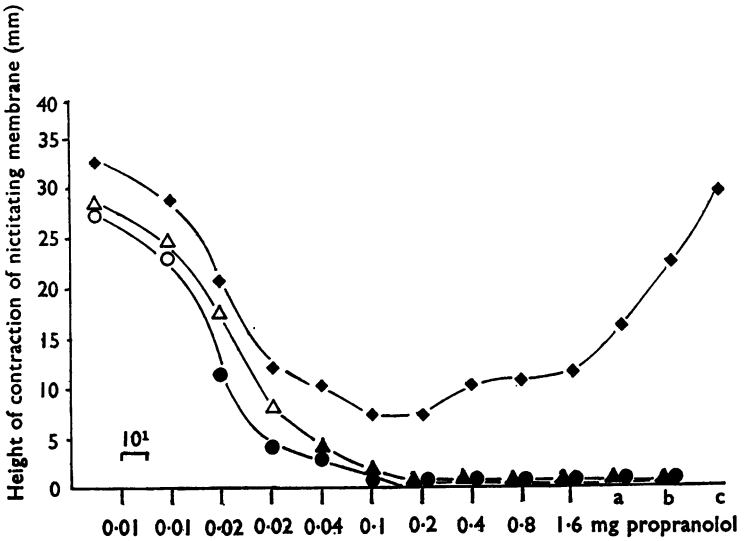

FIG. 1. The effect of propranolol on contractions of the cat nictitating membrane in response to nerve stimulation. ◆——◆, Normal cat. △——△, Cat 3 h after adrenal vein ligation. ○——○, Cat 3 h after section of splanchnic nerves. Where the responses (△ & ○) were significantly different (P=0·05) from corresponding ones in normal cats, the symbols have been filled in. Each point denotes contraction of nictitating membrane, 20 min after each dose of propranolol. Points at a, b and c, 40, 60 and 80 min after 1·6 mg propranolol.

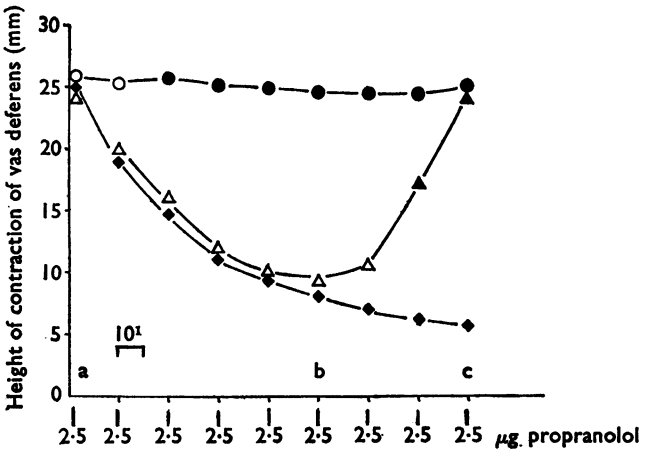

FIG. 2. Antagonism by adrenaline of the adrenergic neurone blocking action of propranolol in isolated vas deferens of the guinea-pig. ◆——◆, Propranolol only from a—c. △——△, Propranolol from a—c, adrenaline 25 μg every 5 min from b—c. ○——○, Propranolol from a—c, adrenaline 25 μg every 5 min from a—c. Where the responses (△ & ○) were significantly different (P=0·05) from corresponding ones in control experiment (◆), the symbols have been filled in. Each point denotes contraction of the vas deferens, 20 min after injection of propranolol. The bath volume was 50 ml so that each injection of 2·5 μg propranolol produced a final concentration of 50 ng/ml.

over the same dose range was also seen in cats in which both splanchnic nerves had been cut 3 h previously (see Fig. 1).

When the splanchnic nerves were severed only 30 min to 1·5 h before propranolol administration, the expected nerve blockade either failed to develop or was very much reduced. It was also greatly diminished in experiments in which adrenaline or noradrenaline was injected in order to exclude a receptor blocking effect of propranolol. These findings suggested that adrenaline or noradrenaline could prevent the nerve blocking action of propranolol.

The antagonistic effect of adrenaline was therefore studied in more detail, by injecting it into normal cats at a dose of 2·5 μg/kg, at 6–8 min intervals between nerve stimulations. In other cats, adrenaline was infused continuously throughout the experiment at the rate of 0·057 (μg/kg)/minute. In both series of experiments, propranolol was injected at 20 min intervals in increasing doses.

In neither type of experiment was any significant degree of nerve blockade observed. In cats with their adrenal veins ligated, infusion of adrenaline before and during the period of nerve stimulation also prevented the nerve blockade by propranolol. In other adrenalectomized cats in which a partial neurone blockade had already been obtained with propranolol, infusion of adrenaline 0·02–0·08 (μg/kg)/minute caused a gradual reversal of the effect in about half the animals tested.

Doses of propranolol, in excess of 100–500 μg/kg given both to normal cats and to cats with adrenal veins ligated, failed to produce significant nerve blockade. Neither did these doses cause any change in the responses of the nictitating membrane to injected noradrenaline.

Isolated guinea-pig vas deferens

Propranolol in concentrations of 25–100 ng/ml produced a reduction in the contraction of the vas deferens in response to stimulation of the hypogastric nerve. Increasing doses up to 500 ng/ml caused no further reduction (see Fig. 2). When the initial dose injected gave a concentration of 200 ng/ml the degree of blockade was either minimal or significantly smaller than that seen with the lower amounts.

As in the cat experiments, the blocking action of propranolol could be prevented by giving adrenaline or noradrenaline before and at regular intervals throughout the experiments (Fig. 2). Once a significant degree of blockade had been established, it was also possible to cause complete reversal by the repeated injection of small doses of adrenaline, which alone, did not produce a contraction of the vas deferens (Fig. 2).

Discussion

It has been shown that the adrenergic neurone blocking action of small doses (10–100 μg/kg) of propranolol could be increased by tying the adrenal veins or by cutting the splanchnic nerves. In the absence of a functional adrenal medulla, the usual reversal of the blockade produced by progressively increasing the dose of propranolol was not obtained.

In the normal cat the action of propranolol on the nerve was prevented when the circulating level of catecholamine was raised by repeated injections or infusions of adrenaline. Increased catecholamine levels probably also account for the failure

of propranolol to produce its expected effect within 30 min to 1·5 h of splanchnic nerve section. After 3 h, however, the more complete blocking action of propranolol was observed, since any adrenaline released by mechanical stimulation of the splanchnic nerve during their isolation and secretion would have been metabolized.

In a proportion of 'adrenalectomized' cats, the response to nerve stimulation could be restored to its control level after blockade by propranolol by a constant infusion of adrenaline. Thus once a source of catecholamine was made available, propranolol was again able to reverse its own blocking action as in the intact cat.

These experiments indicate that reversal of the nerve blockade by propranolol in the intact cat could be obtained only with increasing doses, provided that the adrenal gland was able to secrete catecholamines. Similarly blockade of the hypogastric nerve supplying the isolated vas deferens could be reversed only when exogenous adrenaline was supplied by continuous addition to the organ bath.

The neurone blockade produced by propranolol bears some resemblance to that seen after the administration of bretylium and guanethidine. With each drug the blockade develops slowly after injection and can be prevented or reversed by amphetamine (Day & Rand, 1963; Boura & Green, 1965; Mylecharane & Raper, 1970). There are, however, two obvious differences between the effect of propranolol and that of the conventional neurone blocking agents. The nerve blocking action of propranolol (on the isolated vas deferens preparation) can be reversed or prevented by adrenaline or noradrenaline; but these catecholamines do not antagonize the effect of guanethidine or bretylium (Day, 1962). Secondly, propranolol, but not guanethidine or bretylium, reverses its own action in the whole animal as increasing doses are given. Since this reversal of nerve blockade by propranolol is only demonstrable if secretion from the adrenal medulla is possible, it seems likely that it is due to the release of medullary catecholamines.

It is therefore concluded that propranolol has a potent adrenergic neurone blocking action both *in vivo* and *in vitro*, but this effect may be modified or masked by catecholamine release.

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