Br. J. Pharmac. (1971), **43**, 287–294.

Role of adrenergic neurone blockade in the hypotensive action of propranolol

SARAH ELIASH AND MARTA WEINSTOCK

Department of Physiology and Pharmacology, Tel-Aviv University Medical School, Beilinson Hospital, Israel

Summary

1. Propranolol, in doses of 25–100 μg/kg, blocks contractions of the nictitating membrane to nerve stimulation but not to injected noradrenaline.

2. This adrenergic neurone blocking action of propranolol is antagonized by amphetamine.

3. It is also reversed by raising the dose of propranolol to amounts exceeding 0·5 mg/kg.

4. Still larger amounts potentiate the responses of the nictitating membrane to both submaximal stimulation of the cervical sympathetic nerve and to injected noradrenaline.

5. The (+) isomer of propranolol produced adrenergic nerve blockade and some degree of hypotension without blocking cardiac β-adrenoceptors.

6. The relevance of adrenergic neurone blockade to the hypotensive effect of propranolol is discussed.

Introduction

The β-adrenoceptor blocking agent, propranolol, has been used for the treatment of hypertension with variable clinical success (Pritchard & Gillam, 1969; Zacharias & Cowan, 1970). The exact mechanism by which propranolol exerts its hypotensive action is not clear, but may depend upon the conditions under which it is used.

In hypertensive patients, propranolol reduces blood pressure only after prolonged treatment and it has been postulated that its action results from a resetting of the baroreceptors which then regulate blood pressure at a lower level (Pritchard & Gillam, 1969). In renal hypertensive dogs and rats no fall in blood pressure was achieved with relatively large doses of the drug in both acute and long term experiments. It was, however, possible to reduce blood pressure in normal dogs and rats with propranolol (Farmer & Levy, 1968).

Other workers suggest that the hypotensive effect results from a blockade of β-adrenoceptors in the heart which causes a reduction in cardiac output (Frohlich, Tarazi, Dunstan & Page, 1968; Dorph & Binder, 1969).

Another possibility exists which has not been demonstrated successfully *in vivo*. In several isolated organ preparations, propranolol blocks adrenergic neurones presynaptically in a manner similar to that of guanethidine (Day, Owen & Warren, 1968; Mylechrane & Raper, 1970; Barrett & Nunn, 1970). However, Raper & Wale

(1969) did not find any reduction by propranolol of the effects of pre- or post-ganglionic stimulation of the superior cervical nerve in the cat.

While studying the effects of β-adrenoceptor blocking agents in the cat we were able to demonstrate presynaptic blockade of nerve stimulation by propranolol, but with much smaller doses than those used previously by other workers. In this study we have investigated the conditions under which propranolol produces presynaptic blockade in sympathetic nerves *in vivo*. At the same time an attempt has been made to determine the relevance of such an adrenergic blocking action to the hypotensive effect of the drug.

Methods

Male and female cats weighing 2–3·5 kg were anaesthetized with ether and chloralose, 75–80 mg/kg. The left superior cervical nerve was stimulated pre- or postganglionically at 10 Hz, with a duration of 0.5 ms for 15 s every 2 minutes. Contractions of the nictitating membrane were recorded by means of a Grass force displacement transducer FTO3B on a multichannel polygraph. Injections were made through a cannula in the femoral vein and arterial blood pressure was recorded with a Statham blood pressure transducer attached to a cannula in the femoral artery (1 mmHg≡1·333 mbar).

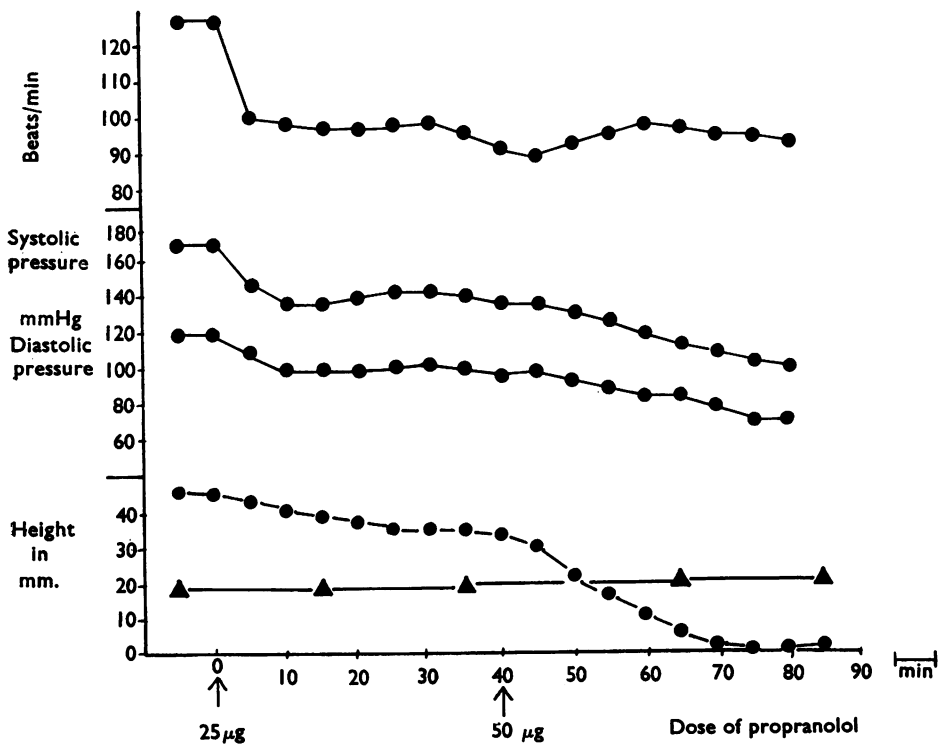

FIG. 1. Effect of propranolol on heart rate, blood pressure and sympathetic nerve stimulation in the cat. Upper record, heart rate in beats/min; middle record, systolic and diastolic blood pressure; lower record, height of contraction of nictitating membrane produced by nerve stimulation ●——●, and injected noradrenaline ▲——▲. (±)-Propranolol injected at arrows.

The drugs used were (±) propranolol hydrochloride (Deralin, Abic Ltd.) (+) propranolol hydrochloride, noradrenaline bitartrate, isoprenaline sulphate and dexamphetamine sulphate. The drugs were dissolved in 0·9% w/v sodium chloride before injection. All doses are expressed in weight per kg body weight of the salt.

Results

The injection of propranolol in doses of 25–100 μg/kg caused a gradual decline in the responses of the nictitating membrane to both pre- and postganglionic stimulation. The maximum reduction usually occurred between 20 and 40 min after injection. These doses of propranolol almost always caused a reduction in heart rate and also antagonized the vasodepressor and cardiac stimulant responses to isoprenaline. The responses of the nictitating membrane or blood pressure to injected noradrenaline were not affected by these amounts of propranolol. The β-adrenoceptor blocking action of propranolol which was often accompanied by a fall in blood pressure, occurred within 5 min of injection, well before the onset of adrenergic neurone blockade (See Fig. 1).

In a few experiments the initial depressor effect was short lived and was followed some time later by a secondary fall in blood pressure which coincided with the development of nerve blockade. The mean fall in blood pressure of fifteen cats given doses of 25–50 μg/kg was 30·5 ± 8·8 mmHg.

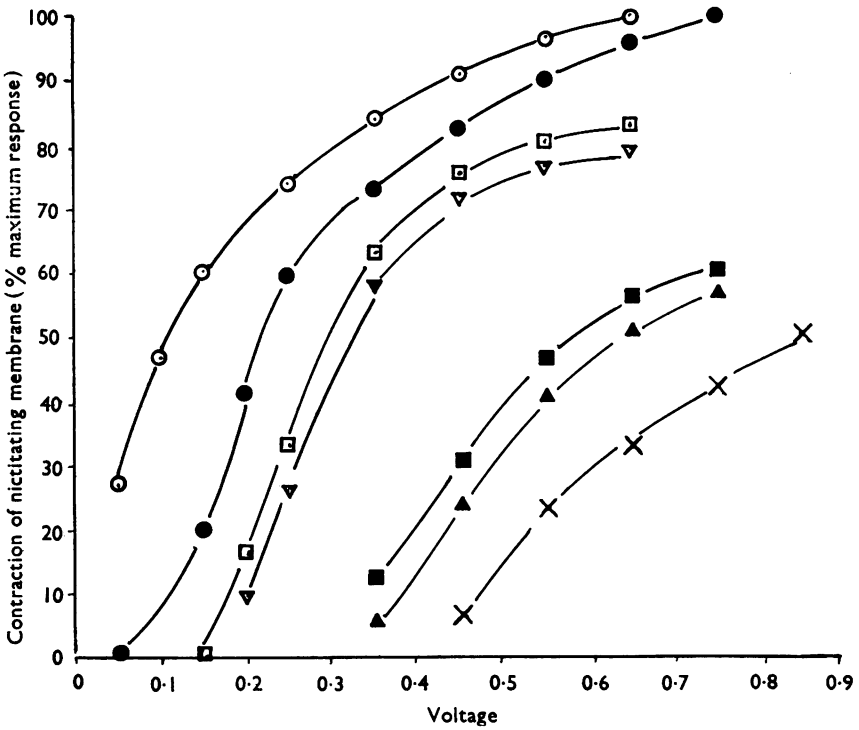

FIG. 2. Effect of propranolol on the contraction of the nictitating membrane elicited by stimulation of the nerve at different voltages. Doses of propranolol: ○——○, 400 μg/kg; □——□, 200 μg/kg; ■——■, 100 μg/kg; ▲——▲, 80 μg/kg; ×——×, 50 μg/kg; ▽——▽, 20 μg/kg; ●——●, control.

In other experiments in which the initial doses of propranolol given were higher, starting with 0·25–0·4 mg/kg, no consistent reduction in the responses to nerve stimulation was achieved. The blood pressure was, however, reduced in almost all animals given these doses, but the average fall in eight cats was only 18·5±8.5 mmHg. This was significantly less than that obtained with the smaller doses which caused adrenergic neurone blockade.

The heart rate always fell after injection of the larger amounts of propranolol but not to a greater extent than with the lower doses of the drug. The responses of the nictitating membrane to injected noradrenaline were usually enhanced.

A series of experiments was carried out in order to determine why propranolol reduced the effects of nerve stimulation at low doses but not at higher ones. The superior cervical nerve was stimulated at a constant frequency and rate with increasing voltages and a voltage-response curve was constructed. Propranolol was injected and the voltage-response curve redetermined continuously by stimulating the nerve every 2 min until no further change in the height of contractions was seen. Further doses of propranolol were injected and voltage response curves redetermined in this way approximately every 25–40 min after injection. In this way it was possible to show both antagonism and potentiation of the effects of nerve stimulation by propranolol. The results of such an experiment are shown in Fig. 2.

The responses elicited by nerve stimulation at the lower voltages were blocked completely by small doses of propranolol, and the contractions produced with higher voltages were correspondingly reduced. Contractions of the original height could be

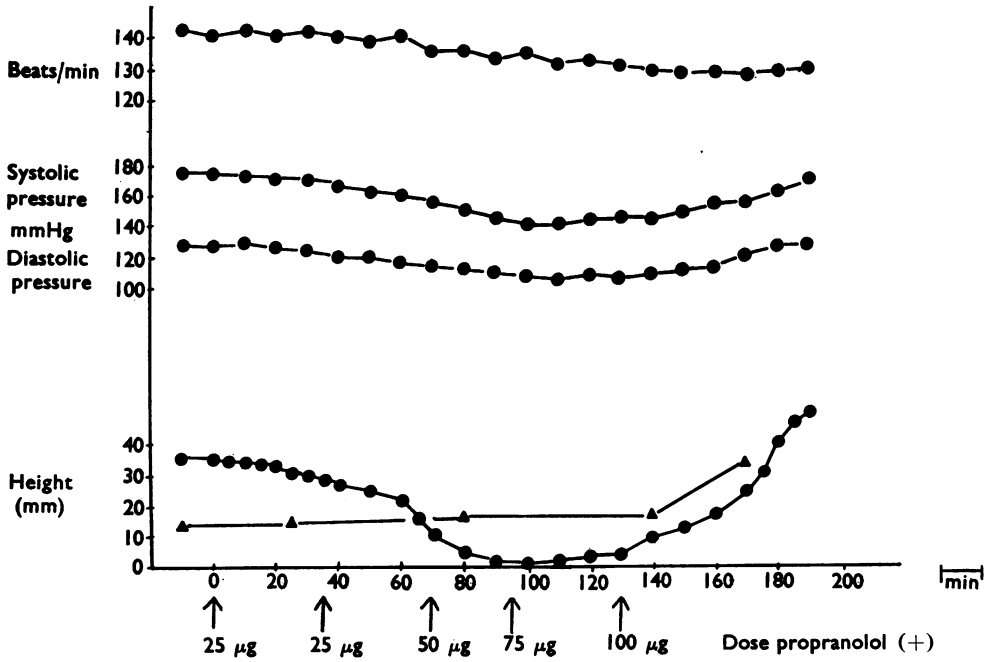

FIG. 3. Effect of (+) isomer of propranolol on heart rate, blood pressure and synpathetic nerve stimulation. Upper record, heart rate in beats/min; middle record, systolic and diastolic blood pressure; lower record, height of contraction of nictitating membrane produced by nerve stimulation ●——●, and injected noradrenaline ▲——▲. (+) Propranolol injected at arrows.

achieved by raising the voltage. A point was reached at which no further reduction in the response of the nictitating membrane could be obtained by increasing the dose of propranolol. Indeed, the responses did not become smaller but gradually returned to their former size and then became potentiated. At this stage it was possible to elicit contractions with lower voltages than those that had been used in the control voltage response curve (Fig. 2). The response of the nictitating membrane to injected noradrenaline was now also potentiated. The effect of isoprenaline on the heart and blood pressure remained completely blocked at all doses of propranolol injected.

Studies with (+) propranolol

In a few experiments the (+) isomer of propranolol was used and found to be approximately equiactive with the racemic mixture in producing presynaptic blockade. Unlike the (±) compound, (+) propranolol antagonized the effects of nerve stimulation at doses which did not block the effects of isoprenaline on β-adrenoceptors. It was therefore possible to demonstrate the relationship between the hypotensive effect of the drug and adrenergic nerve blockade by using the (+) isomer, since no significant change in heart rate occurred. As with the racemic mixture, nerve blockade was produced by small doses and reversed when larger amounts of the drug were injected.

Figure 3 shows the effect of (+) propranolol in increasing doses on contractions of the nictitating membrane, heart rate and blood pressure. The maximal fall in blood pressure coincided with the greatest degree of adrenergic nerve blockade. The blood pressure returned to its control level when the dose was raised and the action of propranolol on the nerve was reversed.

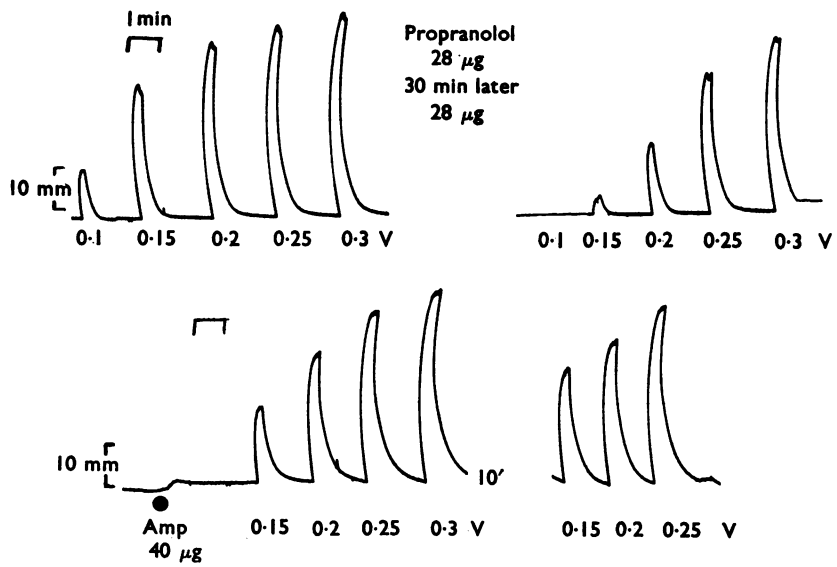

FIG. 4. Antagonism of adrenergic neurone blocking action of propranolol by amphetamine (Amp). Contractions of nictitating membrane produced by preganglionic stimulation of the superior cervical nerve at 10 Hz, 0·5 ms duration. Upper record: responses to nerve stimulation before and 1 h after propranolol given in two doses of 28 μg/kg. Lower record: continuation of upper record showing response to nerve stimulation immediately after amphetamine and after a further 10 minutes.

Antagonism of the effect of propranolol on the nerve by amphetamine

A control-voltage response curve was obtained as described above and propranolol was injected. When a significant reduction in the contractions was achieved, dexamphetamine was given in a dose which itself produced only a very small contraction of the nictitating membrane. Nerve stimulation was then repeated and it was found that amphetamine rapidly reversed the nerve blockade (See Fig. 4).

Discussion

It was possible to block the effect of sympathetic nerve stimulation in the whole animal with propranolol. Significant blockade can be achieved with doses of 25–100 μg/kg given intravenously. Assuming that the drug is distributed rapidly in the extracellular fluid, this amount should provide a local tissue concentration of approximately 0·05–0·2 μg/ml in the cat. These values are the same as the concentrations found by Mylechrane & Raper (1970) to block sympathetic nerves in the isolated guinea-pig vas deferens. Like these authors we found the blockade produced by propranolol to be slow in onset, resembling that of guanethidine. We were also able to reverse the effect of propranolol on the nerve with amphetamine. This finding indicated a further similarity to the blocking action of guanethidine.

The presynaptic action of propranolol differs from that of guanethidine, however, in being incomplete and reversible by increasing the amount of drug injected. When a supramaximal stimulus was used it was only possible to reduce the contractions of the nictitating membrane by at most, 20%. No further blockade could be produced by raising the dose. Contractions elicited by a submaximal stimulus could, however, be markedly reduced by small amounts of propranolol. By using only submaximal stimuli we were able to demonstrate the reversibility of the prejunctional action of propranolol with increasing amounts of the drug. A dose was reached which actually potentiated the contractions of the nictitating membrane produced both by nerve stimulation and by injected noradrenaline. These findings explain the failure of Raper & Wale (1968) to demonstrate a presynaptic blocking action of propranolol in the same preparation, since they used both a supramaximal stimulus and large doses of propranolol, 15 mg/kg.

The results obtained in our experiments confirm and extend the observations of Mylechrane & Raper in demonstrating a dual action of propranolol on adrenergic neurones. At low concentrations the drug prevents the release of noradrenaline from sympathetic nerve endings, presumably if it acts like guanethidine, by being taken up into the storage site normally occupied by the transmitter and released in its place (Chang, Costa & Brodie, 1965; Boullin, Costa & Brodie, 1966).

The second action on the nerve which appears to predominate at doses exceeding 0·5 mg/kg, resembles that of amphetamine and cocaine. Thus propranolol reverses its own blocking action, and even potentiates the responses to nerve stimulation and exogenous noradrenaline in a manner reminiscent of these two drugs. Such an action could result from an interference with the reuptake process for noradrenaline in sympathetic nerves. If propranolol thereby prevents its own uptake into the nerve, the presynaptic blocking action would gradually disappear, to be replaced by increased responses to nerve stimulation as the concentration of noradrenaline available for combination with receptors rises. The ability of propranolol to prevent

the reuptake of noradrenaline in the isolated heart has been demonstrated by Foo, Jowett & Stafford (1968). The concentration required to cause a significant reduction in the uptake of noradrenaline was higher than that needed to block nerve stimulation and was of the order of 1 μg/ml. Assuming that propranolol can interfere with the uptake into nerve and heart muscle equally well, such a concentration could be achieved in the extracellular fluid from doses of 0·5–1 mg/kg.

A fall in both systolic and diastolic blood pressure occurred in all animals given doses of propranolol, which caused appreciable adrenergic neurone blockade. Since the heart rate was also reduced by an average of 15% with these doses, it was not possible to say with certainty that the hypotension resulted from an action on sympathetic nerves and not from blockade of cardiac β-adrenoceptors. However, the fall in blood pressure seen in those animals given larger doses of propranolol, which did not cause nerve blockade, was significantly less, even though the fall in heart rate was at least as great.

In an attempt to define the relative contributions made by blockade of cardiac β-adrenoceptors on the one hand, and reduction of sympathetic nervous activity on the other, experiments were carried out using the (+) isomer of propranolol. This compound was virtually devoid of an action on β-adrenoceptors at doses which caused adrenergic neurone blockade. Thus no significant fall in heart rate was seen, but the blood pressure fell as the response to nerve stimulation was reduced. Furthermore, as the dose of (+) propranolol was increased and the blockade reversed, the blood pressure returned to its control level.

The extent of the fall in blood pressures achieved with the (+) isomer was less than that usually obtained with the racemic mixture. This indicates that propranolol can lower blood pressure by a combined effect on sympathetic nerve and on cardiac β-adrenoceptors. The varying ability of this drug to produce hypotension in experiments of other workers or in human subjects may be explained by the fact that the drug can reverse its own adrenergic neurone blocking action.

The authors wish to thank Professor A. Atsmon for his helpful criticism of this study, and Dr. H. A. Johnston of Medical Services Dept., ICI Ltd., England for supplying (+) propranolol. The able technical assistance of Mrs. Rivkah Coren is also gratefully acknowledged.

This work is part of a MSc thesis to be submitted by S. E. to the Tel-Aviv University Medical School.

REFERENCES

BARRETT, A. M. & NUNN, B. (1970). Adrenergic neurone blocking properties of (±) propranolol and (−) propranolol. *J. Pharm. Pharmac.*, **22**, 806–810.

BOULLIN, D. J., COSTA, E. & BRODIE, B. B. (1966). Discharge of tritium-labelled guanethidine by sympathetic nerve stimulation as evidence that guanethidine is a false transmitter. *Life Sci.*, **5**, 803–808.

CHANG, C. C., COSTA, E. & BRODIE, B. B. (1965). Interaction of guanethidine with adrenergic neurones. *J. Pharm. exp. Ther.*, **147**, 303–312.

DAY, M. D., OWEN, D. A. A. & WARREN, P. R. (1968). An adrenergic neuron blocking action of propranolol in isolated tissues. *J. Pharm. Pharmac.*, **20**, Suppl. 130s–134s.

DORPH, S. & BINDER, C. (1969). Evaluation of the hypotensive effect of beta-adrenergic blockade in hypertension. *Acta med. scand.*, **185**, 443–445.

FARMER, J. B. & LEVY, G. P. (1968). A comparison of some cardiovascular properties of propranolol, MJ 1999 and quinidine in relation to their effects in hypertensive animals. *Br. J. Pharmac.*, **34**, 116–126.

FOO, J. W., JOWETT, A. & STAFFORD, A. (1968). The effects of some β-adrenoreceptor blocking drugs on the uptake and release of noradrenaline by the heart. *Br. J. Pharmac.*, **34**, 141–147.

FROHLICH, E. D., TARAZI, R. C., DUNSTAN, H. P. & PAGE, I. H. (1968). The paradox of beta-adrenergic blockade in hypertension. *Circulation*, **37**, 417–423.

MYLECHRANE, E. J. & RAPER, C. (1970). Prejunctional actions of some β-adrenoreceptor antagonists in the vas deferens preparation of the guinea-pig. *Br. J. Pharmac.*, **39**, 128–138.

PRITCHARD, B. N. C. & GILLAM, P. M. S. (1969). Treatment of hypertension with propranolol. *Br. med. J.*, **1**, 7–16.

RAPER, C. & WALE, J. (1968). Sympathetic involvement in vagal escape and the effects of β-receptor blocking drugs. *Eur. J. Pharmac.*, **8**, 47–57.

ZACHARIAS, F. J. & COWAN, K. J. (1970). Controlled trial of propranolol in the treatment of hypertension. *Br. med. J.*, **1**, 471–474.

(*Received May* 1, 1971)