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THE EFFECT OF PROPRANOLOL ON VASCULAR RESPONSES TO SYMPATHETIC NERVE STIMULATION

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1 In an attempt to clarify the rôle of the sympathetic neurone in the antihypertensive action of propranolol, the effect of this drug on responses to lumbar sympathetic nerve stimulation has been studied in the perfused hind-limb of the dog.

2 No consistent reduction of maximal or submaximal responses to nerve stimulation was produced by propranolol (10 to 100 μg/kg). In contrast, potentiation of nerve-evoked responses, as well as those to injected noradrenaline, usually occurred. Dexpropranolol (50 μg/kg) had no effect.

3 When neuronal uptake of noradrenaline was inhibited by desmethylimipramine or cocaine, no reduction in responses to sympathetic nerve stimulation was observed with propranolol.

4 No evidence was found, using α-adrenoceptor blocking drugs, that released transmitter stimulates β-adrenoceptors in the blood vessels of the hind-limb.

5 No evidence has been found for the existence of an adrenergic neurone-blocking action of propranolol that might contribute to the antihypertensive activity in man.

Introduction

Several hypotheses have been advanced for the antihypertensive action of propranolol. Prichard & Gillam (1969) postulated that the hypotensive effect resulted from a resetting of the baro-receptors, caused by reduction of transient increases in blood pressure of cardiac origin, which thereby regulate the blood pressure at a lower level. Other workers have suggested that a reduction in cardiac output is the primary cause of the antihypertensive effect (Frohlich, Tarazi, Dustan & Page, 1968; Dorph & Binder, 1969) or that decrease of renin release is important (Bühler, Laragh, Vaughan, Brunner, Gavras & Baer, 1973). Some *in vitro* studies have indicated that a pre-junctional block of sympathetic nerve activity may be produced by propranolol, although there is disagreement as to its mechanism and to its relevance *in vivo* (Day, Owen & Warren, 1968; Mylecharane & Raper, 1970; Barrett & Nunn, 1970). Recently, Eliash & Weinstock (1971) reported that propranolol in low doses reduced contractions of the nictitating membrane of cats by adrenergic neurone-blockade whereas high doses had no effect or caused potentiation. Raper & Wale (1969) also indicated that propranolol in high doses had no inhibitory effect in this preparation. Since responses of non-vascular tissue were employed in these findings, extrapolation to nerves innervating vessels may not be valid.

In addition to possible effects on nerves, propranolol also blocks vascular β-adrenoceptors, an action which could contribute to potentiation of sympathetic nerve-evoked pressor responses. However, β-receptors which mediate vasodilatation are believed not to be activated by noradrenaline released from nerves (Glick, Epstein, Wechsler & Braunwald, 1967; Brick, Hutchison & Roddie, 1967), although Viveros, Garlick & Renkin (1968) obtained activation of β-receptors during nerve stimulation.

We have investigated the effect of propranolol, in a range of doses, on vascular responses to sympathetic nerve stimulation and to injected noradrenaline in an attempt to determine whether sympathetic neurones may be involved in the antihypertensive action of propranolol.

Methods

Hind-limb perfusion experiments

Beagle dogs of either sex, weighing 6.5-13.0 kg, were anaesthetized with chloralose (120 mg/kg) injected intravenously and anaesthesia was maintained with chloralose (10 mg/kg) administered every 15 minutes. The left hind-limb was perfused at a constant rate with blood taken from a

cannulated femoral artery, via a roller pump (Watson-Marlow, Model MHRE) and re-introduced distally into the same artery. The limb perfusion pressure was measured by means of a transducer (Bell & Howell, Model 4-327-L221) connected to a T-piece in the inflow tubing. The perfusion rate was adjusted so that the perfusion pressure, which reflects the vascular resistance, equalled the mean systemic arterial blood pressure. The paw circulation was occluded by tight ligatures.

The lumbar sympathetic chain was exposed at the level of L5, ligated and cut. The cut end was placed over bipolar platinum electrodes and stimulated with square-wave pulses of 1 ms duration at a frequency of 10 Hz. The stimulus strength was adjusted to give submaximal or maximal increases in resistance of the perfused limb.

The systemic arterial blood pressure was measured from the right femoral artery and heart rate recorded by means of a cardiotachometer (Horsfall, 1965) using subcutaneous electrodes. The right femoral vein was cannulated for injection of drugs. Drugs were also administered intra-arterially via a needle in the circuit immediately before the pump. Heparin, 1000 units/kg, was injected intravenously before perfusion was started.

Nictitating membrane preparation

Cats of either sex, weighing 1.4-2.1 kg, were anaesthetized with chloralose (80 mg/kg) injected intravenously either with or without induction of anaesthesia with ether. The left nictitating membrane was attached to a strain gauge (Devices Dynamometer UF1) by means of a silk thread. The left cervical sympathetic nerve was stimulated preganglionically via platinum electrodes with supramaximal shocks of 0.5 ms duration at 10 Hz for 15 s every 2 minutes. The isometric contractions of the nictitating membrane were recorded on a Devices M19 recorder. Blood pressure and heart rate were recorded as above.

Drugs

The following drugs were used: atropine sulphate (BDH), cocaine hydrochloride (BDH), desmethylimipramine (Geigy), dibozane [(1,4-benzodioxan-2-yl-methyl) piperazine] (McN-181, McNeil Laboratories), ergotamine tartrate (Sandoz), guanethidine sulphate (Ciba), isoprenaline sulphate (BDH), noradrenaline bitartrate (Burroughs Wellcome), phenoxybenzamine hydrochloride (Smith, Kline and French), propranolol hydrochloride (Inderal, ICI) and dexpropranolol (ICI). In each experiment, atropine sulphate (1 mg/kg) was injected intravenously every hour. All doses are expressed in terms of the salt with the exception of dexpropranolol.

Experiment protocol

Control responses of the hind-limb perfusion pressure to nerve stimulation, isoprenaline and noradrenaline were obtained and the mean for each calculated. Propranolol was then injected intravenously and nerve stimulation and drug injection repeated. The means of responses obtained during a 30 min period after administration of propranolol were compared with the control means, being expressed as percentage change, and the significance of differences determined by Student's t test. For a given dose of propranolol used in at least three different experiments, the mean changes in responses were grouped and the mean obtained.

In the experiments in which α-adrenoceptor blockade was investigated, the blocking drugs were administered intravenously or intra-arterially to the anaesthetized dogs; in the case of phenoxybenzamine the drug was also given to the animal on one or two days before the experiment.

Results

Effects of propranolol

Typical responses to lumbar sympathetic nerve stimulation, and to intravenously injected noradrenaline and isoprenaline on hind-limb resistance, blood pressure and heart rate are shown in Figure 1. The effect of propranolol in one experiment is shown graphically in Figure 2 and the results from all experiments with propranolol (10-100 μg/kg i.v.) are given in Table 1. In only one experiment was there a significant reduction in the maximal nerve-evoked response of the hind-limb resistance following any of the doses of propranolol used. Indeed, a potentiation of maximal responses was usually seen.

The responses to submaximal stimulation were also potentiated except in one experiment (the same one in which the maximal response was reduced) when propranolol 50 μg/kg reduced these responses by 60%. In general, the size of the potentiation was greater with the higher doses of propranolol. The responses to injected noradrenaline were also increased by propranolol which, even in the lowest doses, produced a degree of β-adrenoceptor blockade as judged by the responses to intravenously injected isoprenaline (see Figure 2 and Table 1).

An intravenous dose of guanethidine 5 mg/kg

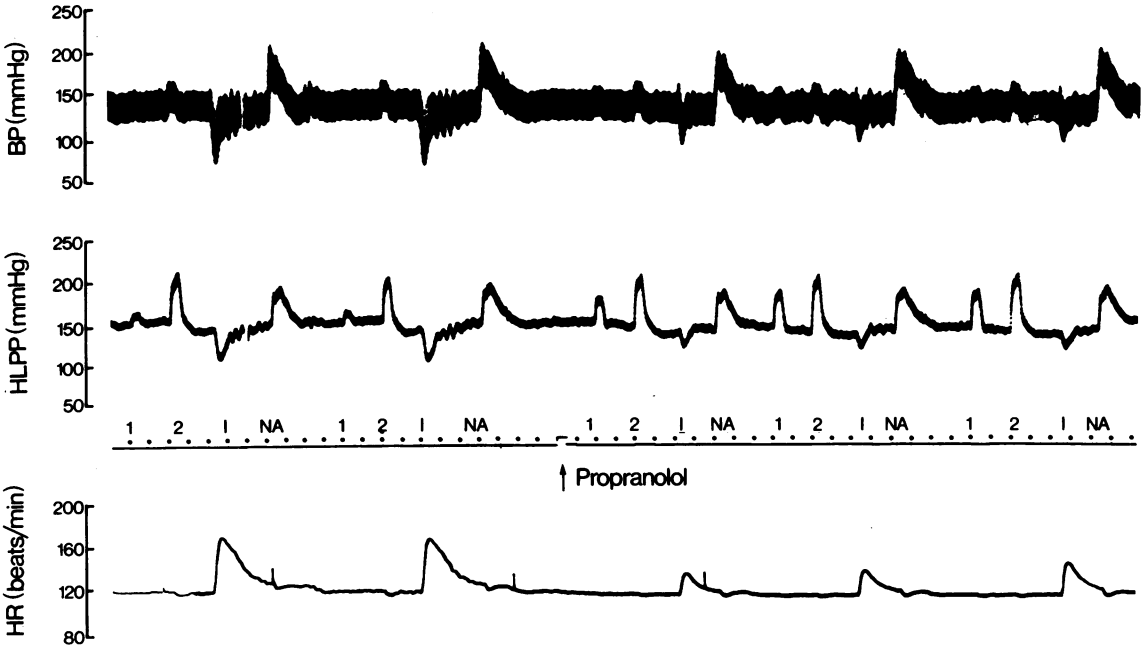

Figure 1 Effect of propranolol (50 μg/kg i.v.) on responses of the blood pressure (BP), hind-limb perfusion pressure (HLPP) and heart rate (HR) to submaximal (1) and supramaximal (2) lumbar sympathetic stimulation, isoprenaline (I, 0.2 μg/kg i.v.) and noradrenaline (NA, 0.2 μg/kg i.v.) in the dog. Time scale: min.

abolished constrictor responses to lumbar sympathetic nerve stimulation within 1 hour. Responses to noradrenaline were enhanced, whilst those to isoprenaline were unchanged.

Inhibition of noradrenaline uptake

It is possible to potentiate responses to sympathetic nerve stimulation and injected noradrenaline by inhibiting the uptake of noradrenaline into neuronal and other sites. This inhibitory effect, leading to increased sympathetic responses, could mask possible neuronal blocking actions of propranolol. Experiments were therefore carried out following the maximal potentiation of responses to nerve-released and injected noradrenaline by desmethylimipramine and cocaine, drugs known to inhibit uptake of transmitter. Pretreatment with these drugs did not modify the enhancement by propranolol of nerve-induced

Table 1 Effect of propranolol, injected intravenously, on responses of the vascular resistance of the perfused dog hind-limb to supramaximal and submaximal lumbar sympathetic stimulation, noradrenaline (0.2 μg/kg i.v.) and isoprenaline (0.2 μg/kg i.v.)

Propranolol (μg/kg)	*No. of expts*	*Effect on responses to:*			
		Submax. stimulation	*Supramax. stimulation*	*Noradrenaline*	*Isoprenaline*
		(Percentage change ± s.e. from mean control responses)			
10	5	+33 ± 11*	+14 ± 5*	+29 ± 9*	−27 ± 7**
25	3	+61 ± 30	+26 ± 10	+66 ± 8	−54 ± 2*
50	5	+76 ± 23*	+27 ± 7*	+31 ± 23	−58 ± 4***
	1	−39	−61	−31	−63
100	4	+97 ± 30*	+23 ± 13	+29 ± 17	−83 ± 3***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

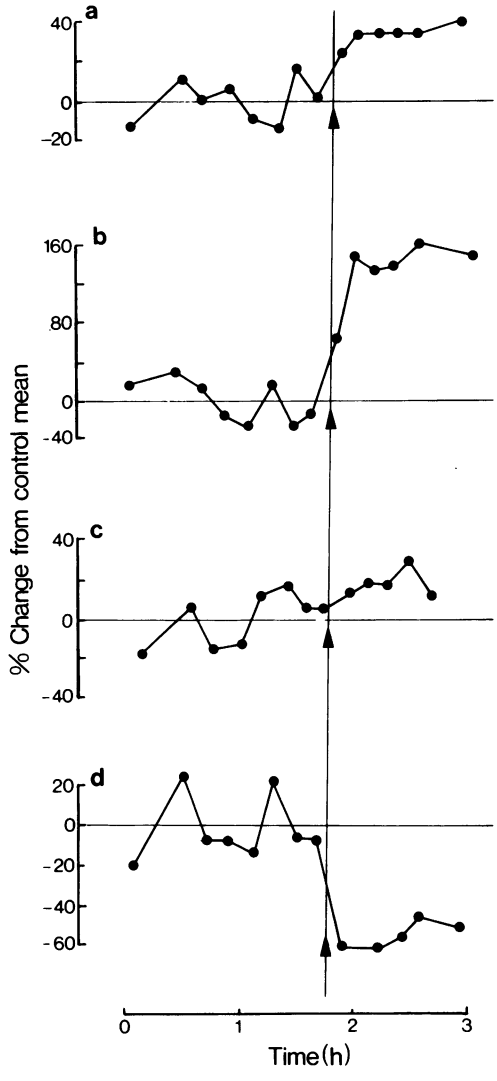

Figure 2 Effect of propranolol, 50 μg/kg i.v. (injected at arrow) on responses of the dog hind-limb perfusion pressure to (a) supramaximal, (b) submaximal lumbar sympathetic stimulation, (c) noradrenaline (0.2 μg/kg i.v.) and (d) isoprenaline (0.2 μg/kg i.v.).

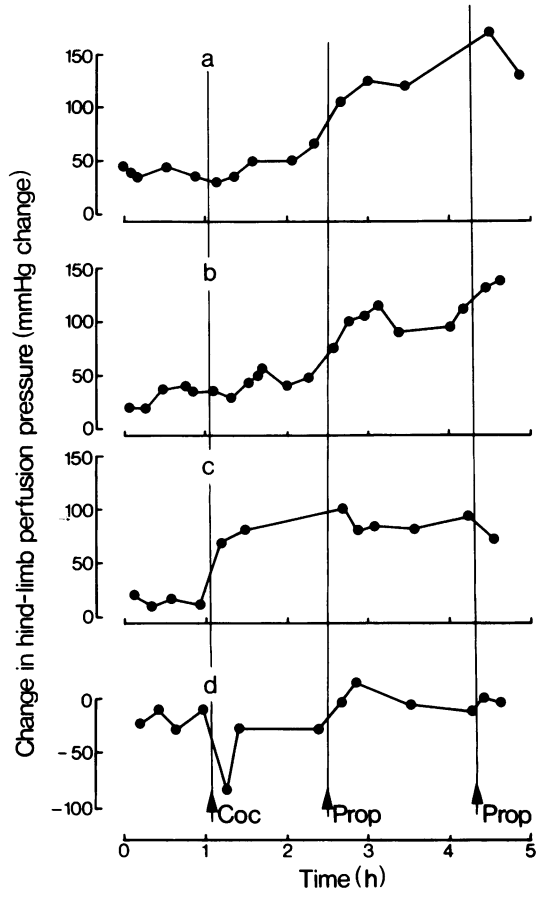

Figure 3 Changes in dog hind-limb perfusion pressure in response to (a) supramaximal and (b) submaximal lumbar sympathetic stimulation, (c) noradrenaline (0.2 μg/kg i.v.) and (d) isoprenaline (0.2 μg/kg i.v.). At the first arrow (Coc), cocaine (3 mg/kg i.v.) was administered and at the second and third arrows (Prop), propranolol (50 μg/kg i.v.) was given.

responses, although no potentiation of responses to injected noradrenaline occurred after propranolol. The hind-limb perfusion pressure increased for about 15 min following the administration of cocaine and then returned to the control base-line where it remained for the duration of the experiment. The resting heart rate did not change following cocaine. Figure 3 shows the effect of propranolol following an intravenous injection of cocaine 3 mg/kg.

Blockade of α-adrenoceptors

If released transmitter activated β-adrenoceptors, which mediate vasodilatation in the skeletal muscle bed, blockade of these receptors would potentiate the constrictor effect. Attempts were therefore made to abolish, by means of α-receptor blocking drugs, the constrictor action of released noradrenaline. If β-receptor activation were involved in the action of the transmitter, a dilatation should occur on nerve stimulation.

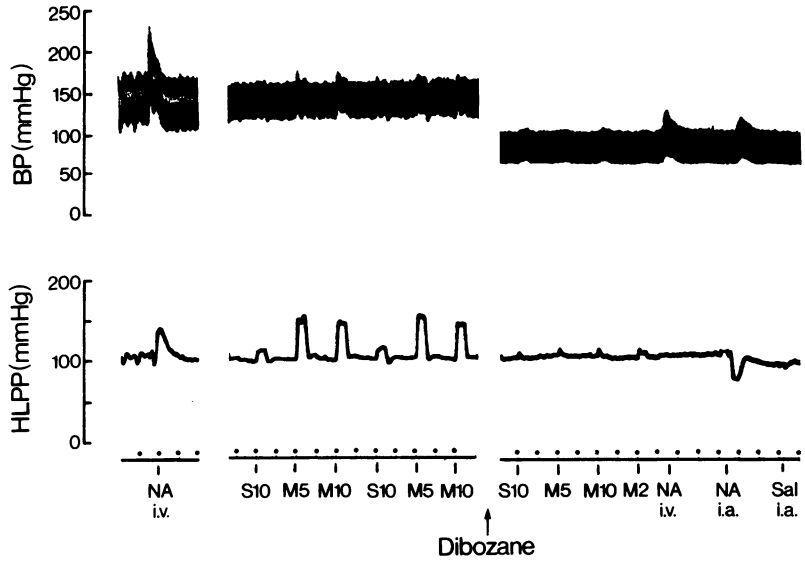

Figure 4 Effect of dibozane (400 μg/min i.a.) on responses of the blood pressure (BP) and hind-limb perfusion pressure (HLPP) to lumbar sympathetic stimulation and noradrenaline (NA 0.5 μg/kg). The nerve was stimulated at submaximal (S) or maximal (M) strength at the frequencies (Hz) indicated by the numbers. Noradrenaline was injected intravenously (i.v.) or intra-arterially (i.a.). An injection of 0.9% w/v NaCl solution (Sal) was also given intra-arterially. The final section of the record was obtained 80 min after starting the infusion of dibozane. Time scale: min.

Phenoxybenzamine (10-20 mg/kg i.v.) reduced the constrictor effect of nerve stimulation but never abolished it. Even by pretreating the animal on three days preceding the experiment (10 mg/kg i.v. per day), small constrictor responses were still evident on stimulation of the lumbar sympathetic nerve. However, by using ergotamine (50 μg/kg i.a. and 200 μg/kg i.v.) in addition to phenoxybenzamine, constrictor responses were abolished, but no dilatation was seen during nerve stimulation.

Using the α-receptor blocker, dibozane (infusion of 400 μg/min i.a.), the constrictor responses were abolished but again no dilatation occurred in response to nerve stimulation (Figure 4), although noradrenaline (0.5 μg/kg) administered intra-arterially into the perfused limb produced vasodilatation. In one experiment in which a combination of phenoxybenzamine and ergotamine were used to produce the α-receptor blockade, vasodilatation was observed on nerve stimulation. However, the responses were unaffected by propranolol (0.03-3 mg/kg i.v.).

Propranolol (0.2 mg/kg i.v.) had little effect on residual constrictor responses during α-receptor blockade but abolished the vasodilatation produced by intra-arterially injected noradrenaline.

Dexpropranolol

Dexpropranolol (50 μg/kg i.v.) had no effect on the responses to lumbar sympathetic nerve stimulation whereas the same dose of racemic propranolol in the same animal exerted the usual potentiating effect.

Experiments on the nictitating membrane of the cat

The experiments of Eliash & Weinstock (1971) were repeated as closely as possible, including induction of anaesthesia with ether before administering chloralose, using isometric contractions of the membrane and employing the same doses of propranolol. In no experiment did propranolol in intravenous doses of 50-150 μg/kg reduce contractions of the nictitating membrane. In five experiments, 50 μg/kg propranolol (the optimal dose for reduction of membrane contractions in the experiments of Eliash & Weinstock) was injected intravenously and the effects on contractions observed for up to 90 min (Figure 5). Although an isoprenaline-induced tachycardia was reduced by the propranolol, no reduction in membrane contractions was seen. Additional

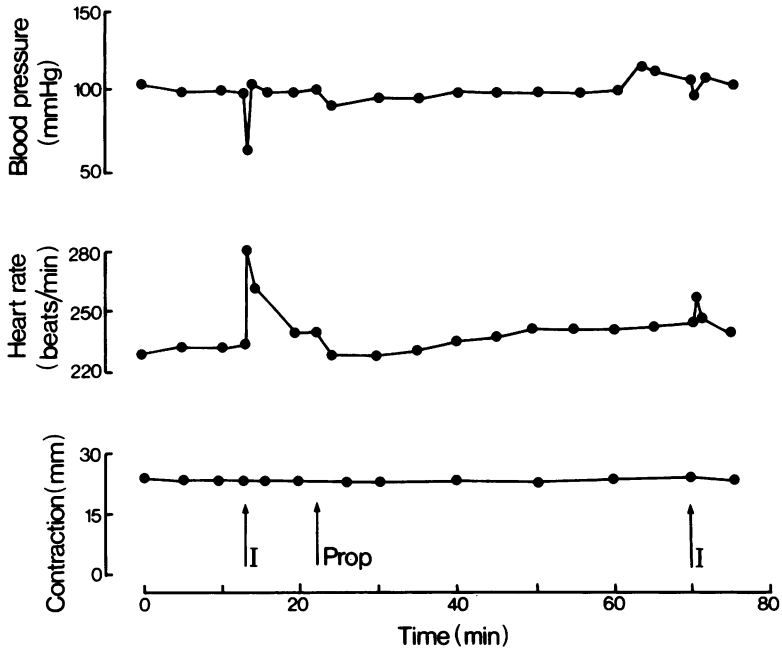

Figure 5 Effect of isoprenaline, 0.1 μg/kg (I) and propranolol, 50 μg/kg (Prop) administered intravenously on the blood pressure, heart rate and size of contractions of the nictitating membrane of an anaesthetized cat.

intravenous doses of 50 μg/kg also did not reduce the magnitude of the contractions. No reduction in blood pressure was produced by these doses of propranolol in our experiments and there was also no potentiation of the responses of the nictitating membrane.

Discussion

In these experiments in atropinized, anaesthetized dogs, using vascular responses elicited by stimulation of sympathetic nerves, no inhibitory effect was produced by propranolol over a 10-fold dose range. In contrast, potentiation was usually seen, even when uptake of released transmitter was inhibited by desmethylimipramine or cocaine. Similarly, Glick *et al.* (1967) and Levin & Beck (1967) observed no inhibition by propranolol of sympathetic constrictor responses and Raper & Wale (1968) did not report any inhibition of nerve-evoked contractions of the nictitating membrane. All these authors used a single dose level of propranolol, usually above 1 mg/kg. However, even with lower doses of propranolol (10 to 100 μg/kg), we have been unable to reproduce the neurone blockade reported by Eliash & Weinstock (1971). These latter authors used the nictitating membrane preparation and extrapolated their findings to resistance vessels in order to explain the antihypertensive action of propranolol. Although various sympathetic nerves might be expected to react in the same manner to neurone-blocking drugs, differences in the end-organ may affect the results. For example, it has been reported that the nictitating membrane is devoid of β-receptors (see Levin & Beck, 1967), or has only weakly reactive ones (Smith, 1963), whereas arteries and arterioles are known to respond readily to β-receptor stimulation. However, even in the nictitating membrane preparation we have been unable to detect a depressant effect of propranolol using nerve stimulation. The reason for a discrepancy between our results and those of Eliash & Weinstock (1971) is not clear. Furthermore, in the experiments reported here, no fall in blood pressure is seen with these low doses of propranolol whereas Eliash & Weinstock (1971) show a marked fall.

Another factor which may influence the results obtained on blood vessels is inhibition of uptake of released noradrenaline. Propranolol in this respect is one tenth as potent as cocaine (Foo, Jowett & Stafford, 1968), and if a preparation were particularly sensitive to block of uptake, then a potentiation to released and injected noradrenaline might mask inhibitory effects. However, even

following treatment with desmethylimipramine or cocaine, no inhibitory effect of propranolol on constrictor responses to sympathetic nerve stimulation was observed. It is possible that the doses of the uptake inhibitors did not completely block re-uptake of nerve-released transmitter and propranolol produced further potentiation by a cocaine-like action. There is no evidence that propranolol can enhance the release of noradrenaline from sympathetic nerves (Starke & Schumann, 1972).

Potentiation of the responses to injected noradrenaline by low doses of propranolol (10-100 μg/kg) could be due to blockade of the β-adrenoceptors which mediate vasodilatation. However, no evidence could be found in these experiments for activation of β-receptors by nerve-released noradrenaline. This is in agreement with the findings of Glick *et al.* (1967) and Brick *et al.* (1967), although Viveros *et al.* (1968) obtained a vasodilatation on nerve stimulation during α-receptor blockade with dibozane.

Eliash & Weinstock (1971) found that higher doses of propranolol (0.5 mg/kg) reversed the adrenergic neurone blockade produced by lower doses (25-100 μg/kg) in the cat and proposed that release of catecholamine from the adrenal medulla was responsible for this effect (Eliash & Weinstock, 1972a, b). However, we saw no change in perfusion pressure that might be indicative of an increased circulating level of catecholamine. There is, therefore, no evidence that adrenal stimulation is more apparent in the dog than in the cat.

Dexpropranolol, which has less than 1% of the potency of propranolol as a β-receptor blocking agent (Howe & Shanks, 1966), did not potentiate or reduce the responses to nerve stimulation. The fact that dexpropranolol is as potent as propranolol in blocking uptake of noradrenaline (Foo *et al.*, 1968), is further evidence that this effect is not responsible for the potentiation by propranolol of nerve-evoked constrictor responses. An effect due to a β-receptor blocking action seems likely, therefore, although evidence of a post-synaptic action on β-receptors has not been obtained.

If these doses of propranolol released adrenal catecholamines (Regoli, 1970) which stimulated the release of renin and thereby angiotensin, an increased noradrenaline release due to angiotensin (Zimmerman, 1962) could be responsible for the potentiation of nerve-evoked responses seen with propranolol. However, significant adrenal catecholamine release seems unlikely with these low doses of propranolol and this β-blocking drug is also known to reduce catecholamine-induced release of renin (Winer, Choksi & Walkenhorst, 1970; Assaykeen, Clayton, Goldfein & Ganong, 1970). Eliash & Weinstock (1971) showed that dexpropranolol also reduced contractions of the nictitating membrane although this drug does not exert an antihypertensive effect in man (Waal-Manning, 1970; Prichard, 1971).

No evidence has therefore been found to support the contention that a guanethidine-like neurone-blocking action of propranolol in low doses might contribute to its antihypertensive effect. Clinically, the antihypertensive dose of propranolol is usually larger than the minimum dose to block β-receptors whereas larger doses were found by Eliash & Weinstock (1971) to reverse the neurone blockade. Moreover, the guanethidine-like side-effects of postural hypotension and failure of ejaculation do not occur with propranolol.

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