Br. J. Pharmac. Chemother. (1966), **28**, 15–22.

MECHANISM OF A SYMPATHOMIMETIC ACTION OF PROPRANOLOL IN DOG

BY

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(*Received April* 21, 1966)

Propranolol was introduced by Black, Crowther, Shanks, Smith & Dornhorst (1964) as a potent beta adrenergic receptor blocking agent. In more detailed studies it was found to be devoid of intrinsic sympathomimetic activity (Black, Duncan & Shanks, 1965 ; Shanks, 1966). Long term treatment with this agent was reported to cause reductions of blood pressure in hypertensive and normotensive patients (Prichard & Gillam, 1964 ; Gillam & Prichard, 1965). These authors attributed this effect of propranolol to interference with the function of the sympathetic nerves to the heart. However, propranolol given intra-arterially was recently shown to produce a sustained vasoconstriction in the perfused hind limb of the dog (Nakano & Kusakari, 1965, 1966).

The present study was undertaken to elucidate the mechanism of the sustained pressor action of propranolol on the sympathetically denervated vasculature in a separately autoperfused hindlimb preparation of the dog described by Kayaalp (1966). Evidence will be given for a catecholamine releasing action of propranolol as the main cause of this pressor effect.

METHODS

Dogs of either sex, weighing between 11 and 25 kg were used. They were anaesthetized by intravenous injection of sodium pentobarbitone (30 mg/kg). The trachea was cannulated for artificial ventilation, and the left jugular vein was cannulated for intravenous injection of drugs. The method for hindlimb perfusion has been described in detail elsewhere (Kayaalp, 1966). Briefly, while the animal was lying on the right side, the aorta was dissected retroperitoneally through an incision in the left flank. The lumber sympathetic chains on both sides were sectioned at mid-lumbar level. In some experiments the sciatic nerves, which are known to convey the majority of the sympathetic nerves from the lumbar sympathetic chain to the hindlimb vasculature (Clonninger & Green, 1955 ; Kayaalp, 1965) were also sectioned. The side branches of lumbar aorta and hypogastric artery were tied off in order to eliminate the anastomoses as much as possible. The aorta was tied and blood from the proximal portion was perfused through each hind limb with two Sigmamotor pumps, one supplying each iliac artery. The rate of the pumps was adjusted so that the initial perfusion pressures were approximately equal to the systemic blood pressure. The perfusion pressures in the hind limbs were recorded on smoked paper from single arm mercury manometers attached to a side arm of the inflow cannula ; the systemic blood pressure was recorded from a double arm mercury manometer attached to a side arm of the cannula inserted into the proximal portion of the aorta. Heparin sodium (5 mg/kg I.V.) was injected at the end of surgery and the dogs were ventilated. In some experiments only one limb was perfused.

After 45 to 60 min the perfusion pressures were stable and the pump rates were readjusted to give perfusion pressures similar to the systemic blood pressure. The pump rates were then kept constant throughout the experiment. Since the output from the pump was constant, changes in perfusion pressure reflected changes in vascular resistance of the perfused area. The completeness of the sympathetic denervation was verified at the beginning of the experiment by the absence of the reflex vasodilatation in the hindlimbs in response to the systemic blood pressure rise induced by noradrenaline (0.5 μg/kg I.V.).

Adrenal gland exclusion. In three dogs, after a mid-line incision of the abdominal wall, the adrenal glands were found at both sides. Their vascular connexions with the surrounding tissue were tied. The glands were left in place. Three hours were allowed to elapse before starting surgery for perfusion of the hind limbs.

Section of spinal cord. In four dogs, the muscles of the neck were dissected to expose the atlanto-occipital membrane, and the spinal cord was then cut through an incision in the membrane. Both common carotid arteries were tied. Three hours were allowed to elapse before beginning the perfusion of the hind limbs.

Drugs used were propranolol hydrochloride (Inderal, I.C.I.), phentolamine hydrochloride (Regitine, Ciba), phenoxybenzamine (Dibenzyline, Smith Kline & French), morphine hydrochloride, (−)-noradrenaline bitartrate (Hoechst) and (−)-adrenaline hydrochloride. Doses of the drugs are expressed as salt, except for the last two, which are expressed as the base.

RESULTS

Effect of propranolol on perfusion pressure

Observations were made in nine dogs. In three, propranolol (0.05 to 0.1 mg/kg) was given intra-arterially to one of the hind limbs. It produced an initial transient fall and then a progressive sustained rise in the perfusion pressure in this side, without any appreciable alteration in the systemic blood pressure. Interestingly, there was a parallel increase in the perfusion pressure of the other leg, where no drug was given. Since the anastomoses between the hind limbs were adequately eliminated (Kayaalp, 1966), these observations suggested that a pressor substance was liberated outside the perfused areas. Consequently propranolol was administered intravenously in four dogs. Propranolol (0.05 to 0.1 mg/kg and/or 1 mg/kg I.V.) elicited a sustained rise in perfusion pressure (Fig. 1). In this experiment, phentolamine (2.5 mg/kg I.V.) was given after the rise induced by propranolol reached a plateau ; the perfusion pressure fell again. A similar fall was obtained after the injection of phenoxybenzamine (3 mg/kg I.V.) in another experiment.

The sustained rise elicited by propranolol was progressive and reached a plateau within 15 to 35 min after administration of the drug. The magnitude of the rise was variable, ranging from 45 to 115 mm Hg and appeared to be independent of the route of administration.

Propranolol (0.05 to 0.1 mg/kg I.V. or I.A.) did not induce any appreciable reduction of the systemic blood pressure. However it usually elicited a slight fall in the blood pressure when given at the dose of 1 mg/kg intravenously.

In two dogs, in which only the left hind limb was denervated, propranolol was injected intra-arterially to the denervated limb ; the perfusion pressure started to rise in the other hind limb after a mean delay of 43 sec. In these experiments, the mean time taken by noradrenaline (0.5 μg/kg) injected into the left jugular vein to reach the hind limb was

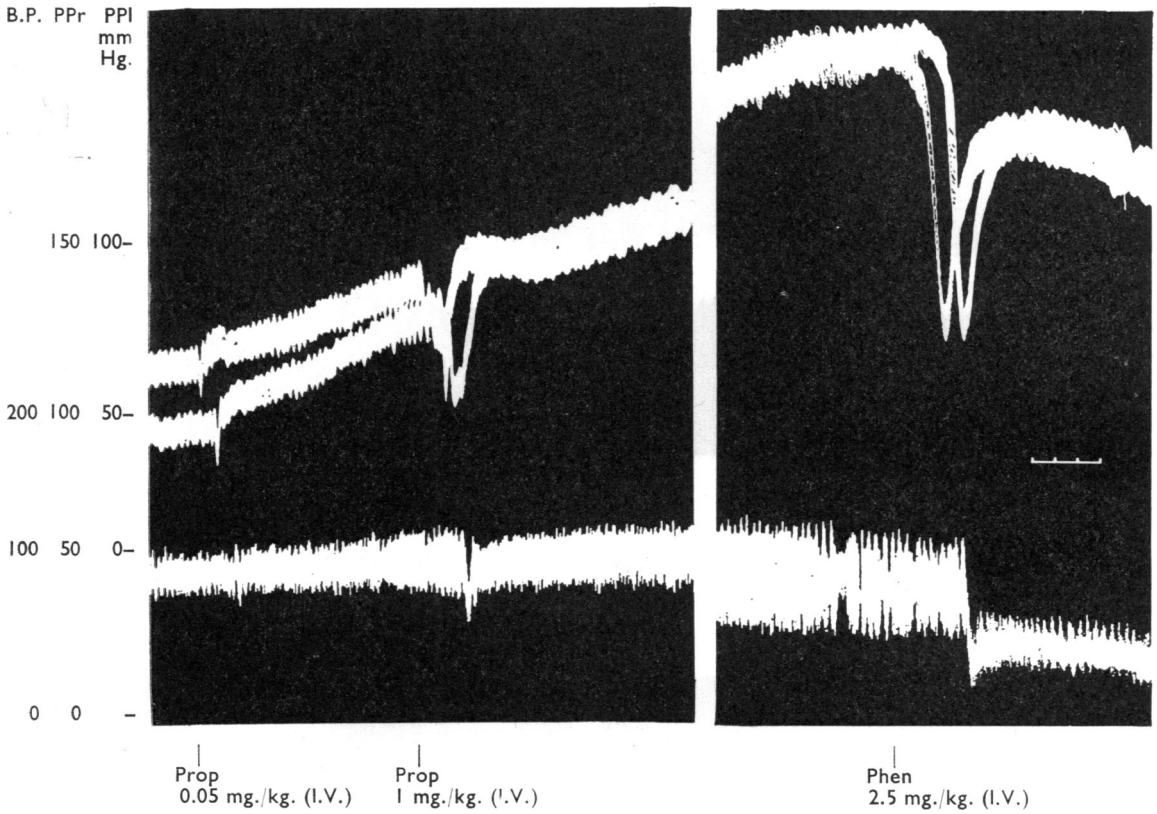

Fig. 1. Dog anaesthetized with pentobarbitone and artificially ventilated, both hind limbs auto-perfused separately. Recordings of blood pressure (B.P., lower), right hind limb perfusion pressure (PPr, middle) and left hind limb perfusion pressure (PP1, upper). Response to propranolol (Prop) and effect of phentolamine (Phen) on this response is shown. Interval between left and right panels is 12 min. Note that perfusion pressure recordings overlapped after second dose of propranolol. Time marks, 1 min.

found to be 21 sec. On the basis of this result, it seemed highly unlikely that the pressor response on the hind limb was due to a reflex sympathetic discharge activated by the stimulation of the sensory nerves in the perfused area by propranolol. Furthermore, in these dogs, the rises in perfusion pressures in both legs appeared to be similar in course and magnitude.

Effect of propranolol in dogs treated with blocking agents for α-receptors of catecholamines

Phentolamine was infused intra-arterially to both hind limbs at a rate of 2.4 to 3 mg/min for 40 min. Alpha-receptor blockade was verified by the abolition of the pressor response to noradrenaline and the reversal of the response to adrenaline given intra-arterially. While the infusion was in progress, propranolol was injected intravenously at the dose of 0.1 mg/kg and subsequently 1 mg/kg. The first dose elicited a minor

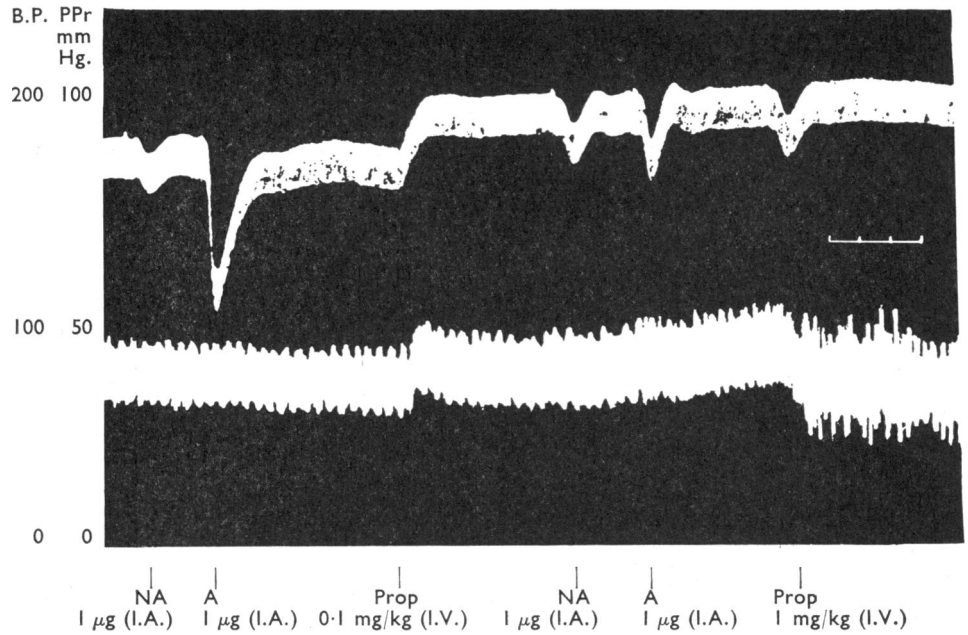

Fig. 2. Dog anaesthetized with pentobarbitone and artificially ventilated, right hind limb auto-perfused, intra-arterial infusion of phentolamine (2.4 mg/min) in progress. Recordings of blood pressure (B.P., lower) and right hind-limb perfusion pressure (PPr, upper). Response to noradrenaline (NA), adrenaline (A) and propranolol (Prop) is shown. Time marks, 1 min.

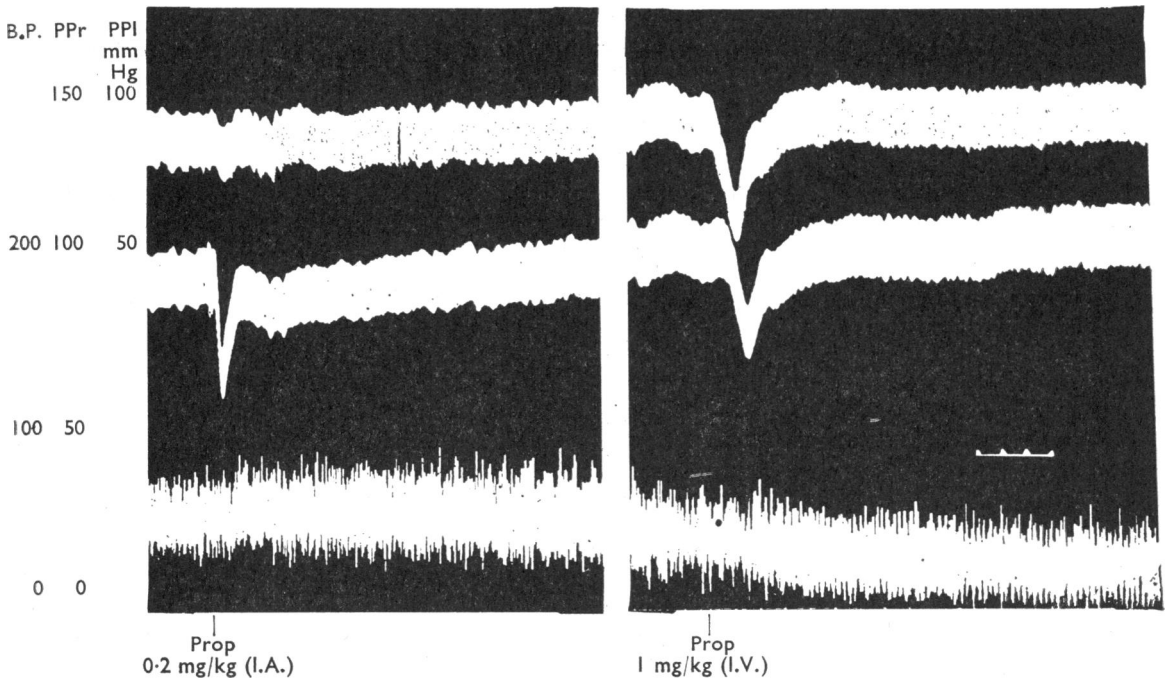

Fig. 3. Dog anaesthetized with pentobarbitone and artificially ventilated, both adrenal glands perfused, intra-arterial infusion of phentolamine (2.4 mg/min) in progress. Recordings of blood pressure (B.P., lower), right hind limb perfusion pressure (PPr, middle) and left hind limb perfusion pressure (PPl, upper). Response to propranolol (Prop) is shown. Interval between left and right panels is 20 min. Time marks, 1 min.

sustained rise (less than 20 mm Hg), which levelled off immediately (Fig. 2). The subsequent higher dose did not elicit any further rise in perfusion pressure. The smaller dose of propranolol caused a slight rise in the systemic blood pressure, but the higher dose produced a fall. Two other dogs were given phenoxybenzamine (10 mg/kg) in slow intravenous infusion for 15 min. One hour after the end of infusion propranolol given in doses up to 1 mg/kg intravenously did not elicit any pressor response in the perfused areas but only a transient dilatation.

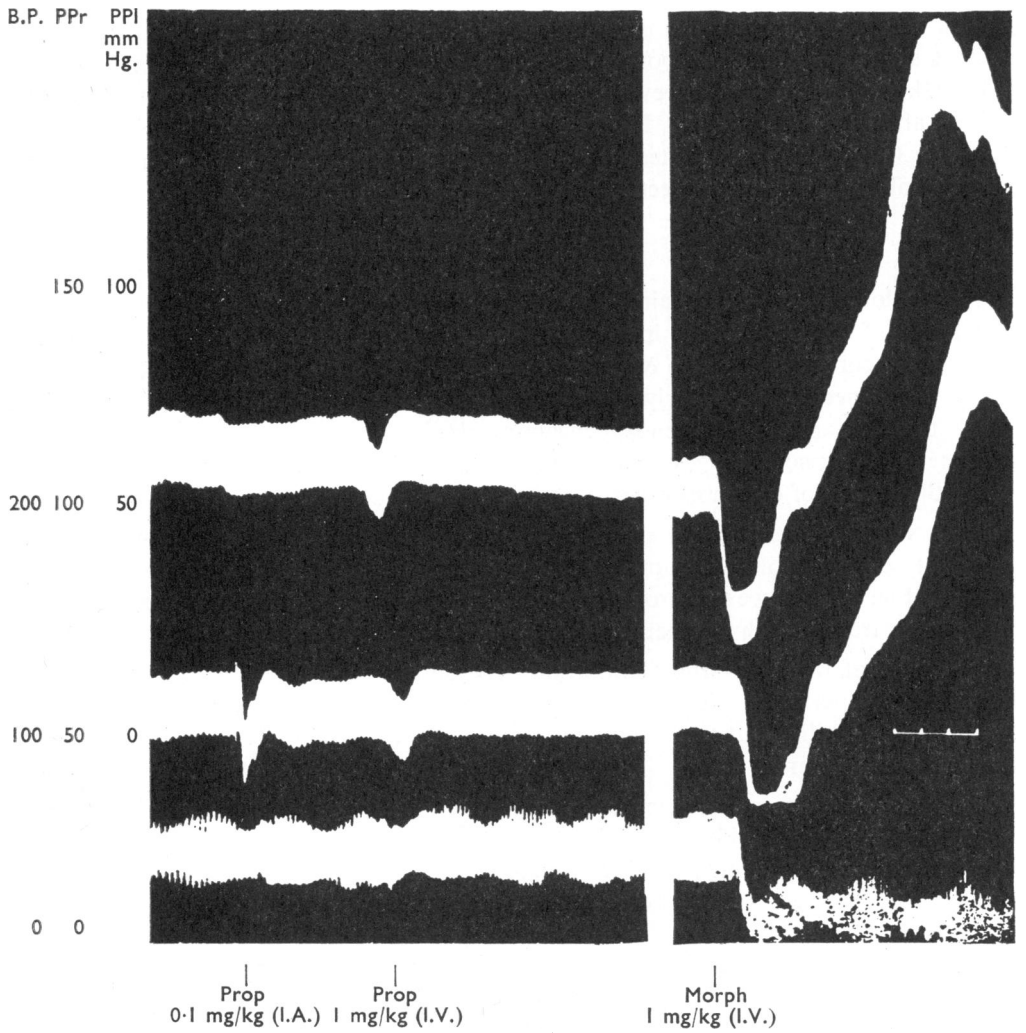

Fig. 4. Spinal dog anaesthetized with pentobarbitone and maintained on artificial ventilation, both hind limbs autoperfused separately. Recordings of blood pressure (B.P., lower), right hind limb perfusion pressure (PPr, middle) and left hind limb perfusion pressure (PPl, upper). Response to propranolol (Prop) and to morphine (Morph) is shown. Interval between left and right panels is 10 min. Time marks, 1 min.

Effect of propranolol in dogs whose adrenals were excluded

Observations were made in three dogs in which the adrenal glands had been excluded from the circulation. Propranolol (up to 1 mg/kg I.A. or I.V.) caused only a transient fall in the perfusion pressures. Figure 3 shows the responses of the perfusion pressures to propranolol (0.2 mg/kg I.A. and 1 mg/kg I.V.).

Effect of propranolol in spinal dogs

In three dogs whose spinal cord was transected, propranolol (up to 1 mg/kg I.V.) did not cause a pressor response in the perfused areas. In one other spinal dog, it produced a slight sustained pressor response of less than 30 mm Hg when given intravenously (0.3 mg/kg). In this dog, however, the completeness of the spinal cord section was not verified after the experiment. Figure 4 shows tracings from an experiment in which morphine (1 mg/kg I.V.) elicited a large sustained rise in the perfusion pressures, but propranolol had no pressor effect.

DISCUSSION

Propranolol produces a sustained vascoconstriction in the sympathetically denervated vasculature of the hind limbs, in addition to a transient vasodilatation. This confirms the results reported by Nakano & Kusakari (1965, 1966). No attempt has been made to elucidate the mechanism underlying the vasodilator response in the present work. The fact that phentolamine and phenoxybenzamine abolished or nearly abolished the pressor response to propranolol suggests that this response is not produced by propranolol *per se* but by the release of catecholamines.

It was surprising to obtain a small sustained rise in the perfusion and systemic blood pressure to propranolol given during alpha adrenergic receptor blockade by phentolamine infusion (Fig. 2). However, propranolol did not cause any sustained pressor response in two dogs treated with another alpha adrenergic receptor blocker, phenoxybenzamine.

The fact that the intra-arterial injection of propranolol to one leg caused a parallel increase in the perfusion pressure of the other leg may be taken as an indirect evidence against the possibility that the pressor response is due to a local release of catecholamine from the perfused area.

The lack of any pressor response to propranolol in dogs whose adrenal glands were excluded indicates that this response was due to a release of catecholamines from the adrenal medulla by propranolol. The failure of propranolol to elicit a pressor response in spinal dogs suggests that the catecholamine releasing action of propranolol from the adrenal medulla is due to a reflex mechanism. A fall of the systemic blood pressure causes release of catecholamine from the adrenal medulla, though the extent to which the adrenal medulla takes part in the sino-aortic baroreceptor reflex seems to be small (Heymans & Neil, 1958). The following indirect evidence appears to be against the involvement of such a reflex mechanism in the catecholamine release by propranolol from the adrenal medulla: (1) propranolol given at the lower dose usually did not produce any fall in the systemic blood pressure but did produce a large sustained pressor response in the perfused areas; (2) pronethalol and dichloroisophoterenol, two other beta adrenergic blocking agents, did not produce a pressor response in the similarly perfused

hind limbs of the dogs whereas they caused a marked sustained fall in the systemic blood pressure (Kayaalp, 1965).

Another possibility regarding the nature of the catecholamine releasing action of propranolol from the adrenal medulla, which cannot be ruled out on the basis of the results obtained in the present work, might be that this action requires an intact sympathetic flow from the central nervous system to the adrenal medulla. Consequently a connexion with and presumably stimulation by the central nervous system might be essential for this action of propranolol. Evidence was given for such a mechanism of catecholamine releasing action of reserpine in cats and rabbits (Holzbauer & Vogt, 1956 ; Kroneberg & Schümann, 1957 ; Muscholl & Vogt, 1958), and of insulin in various species (Garrett, 1965).

Although propranolol increased the pressure in the perfused areas, it usually caused a fall in the systemic blood pressure when given at higher dose levels. This discrepancy can be explained, at least partly, on the basis of the fact that the depressant action of this drug on the heart arising from the beta-receptor blockade (Black *et al.*, 1964, 1965 ; Nakano & Kusakari, 1965, 1966 ; Shanks, 1966) can overcome the pressor action of the catecholamines released.

SUMMARY

1. Propranolol induced a transient vasodilation followed by a sustained vasoconstriction in the denervated autoperfused hind limbs of the dogs. The mechanism of the pressor response to propranolol was investigated.

2. The alpha adrenergic blocking agents, phentolamine and phenoxybenzamine, reduced or abolished the pressor response to propranolol. Propranolol did not produce any pressor response in spinal dogs, or in dogs whose adrenal glands were excluded from the circulation.

3. These results suggest that the pressor response to propranolol is due to the release of catecholamines from the adrenal medulla, which may be related to a reflex activation. However, indirect evidence does not support a reflex action of propranolol. Another possibility is that the release of catecholamines by propranolol may have a connexion with the central nervous system and require an intact sympathetic flow to the adrenal medulla.

The authors wish to thank Mr. R. Sarihüseyinoglu and Mr. M. Kabaçam for their skilled technical assistance. Our thanks are also due to the firms who supplied us with propranolol (I.C.I.), phentolamine (Ciba), phenoxybenzamine (Smith Kline & French) and noradrenaline (Hoechst). This investigation was carried out with the aid of NATO Research Grant No. 212.

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