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The reversal of phenoxybenzamine-produced α-adrenoceptor blockade by the isomers of propranolol and INPEA

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Isomers of propranolol and *N*-isopropyl-*p*-nitrophenylethanolamine (INPEA) were used to demonstrate that there are two mechanisms by which the β-adrenoceptor blocking agents will reverse phenoxybenzamine-produced α-adrenoceptor blockade. In the seminal vesicle preparation, prior administration of either isomer initially protected the receptors from phenoxybenzamine blockade when the contact time for phenoxybenzamine was short. The isomers were equi-effective, suggesting that this action is independent of β-adrenoceptor blocking activity. When the contact time of phenoxybenzamine was prolonged, the ability of the isomers to protect the α-receptors was lost. In the rat blood pressure preparation, after the development of phenoxybenzamine-produced α-adrenoceptor blockade, (−)- and (±)-propranolol or (−)- and (±)-INPEA, in doses that produced marked β-adrenoceptor blocking activity, partially reversed the α-adrenoceptor blockade. Identical doses of (+)-propranolol or (+)-INPEA, which exhibited weak β-adrenoceptor blocking activity did not produce any reversal. The reversal of phenoxybenzamine-produced α-adrenoceptor blockade in this situation appears therefore to be dependent upon the development of β-adrenoceptor blockade.

The mechanism by which the β-adrenoceptor blocking agents reverse the α-adrenoceptor blocking actions of α-blocking agents has not been fully elucidated. Two hypotheses have been put forward. Gulati, Gokhale & Udwadia (1965), Olivares, Smith & Aronow (1967), Patil, Tye, & others (1968) and Guimaraes (1969) suggest that the β-adrenoceptor blocking agents compete with the α-adrenoceptor blocking agents for the α-receptor. On the other hand, Garrett, Malafaya-Baptista & Osswald (1965), Yamamura & Horita (1968) and Smith & Nash (1969) suggest that the reversal of α-adrenoceptor blockade by the β-adrenoceptor blocking agents is due to the blockade of β-receptors resulting in the unmasking of residual α-activity not previously blocked by the α-adrenoceptor blocking agent. This paper presents the results of the effects of the isomers of propranolol and of *N*-isopropyl-*p*-nitrophenylethanolamine on the reversal of phenoxybenzamine-produced α-adrenoceptor blockade and discusses the results in terms of the two hypotheses.

METHODS

Rat blood pressure preparation. Rats (Wistar strain), 160–220 g, were anaesthetized with urethane (100 mg/100g). The trachea was cannulated, the blood pressure monitored from the left carotid artery by means of a Condon manometer, which wrote on a smoked kymograph, and drugs were administered through a polythene

cannula inserted into the left femoral vein. The rats were heparinized with 50 units/100 g and drugs were administered in 0·1 ml volumes and washed through the cannula with 0·1 ml saline.

Reversal of α-adrenoceptor blockade. Responses to noradrenaline, phenylephrine or methoxamine at four dose levels were obtained. Phenoxybenzamine (2·5 mg/kg) was then administered slowly in 0·5 ml of saline and its effect allowed to develop over 60 min, at which time the responses to the agonist were again determined. (−)-, (+)- or (±)-Propranolol (1 mg/kg) or (−)-, (+)-, or (±)-INPEA (10 mg/kg) was then administered slowly in 0·25 ml saline and its effect allowed to develop for 10 min at which time the responses to the agonist were again noted.

β-Adrenoceptor blocking activity. Responses to isoprenaline at three dose levels were obtained. One of the isomers or the racemate of propranolol (1 mg/kg) or of INPEA (10 mg/kg) was administered and its effect allowed to develop for 10 min, at which time the effects of isoprenaline were again determined.

Guinea-pig seminal vesicle preparation

Seminal vesicles were taken from decapitated male guinea pigs (400–800 g) and suspended in a 10 ml organ bath containing Krebs solution gassed with 5% carbon dioxide in oxygen at 37°. The seminal vesicle was attached to a frontal writing lever which wrote on a smoked kymograph. The movements of the tissue were magnified 10 times and the tissue was subjected to a load of approximately 0·5 g. A vibrator was used throughout the experiment to ensure that the vesicle relaxed fully. Two kinds of experiment were made.

Effect of β-adrenoceptor blocking agents on α-adrenoceptor blockade, Responses to noradrenaline or phenylephrine at four dose levels were obtained, using a drug contact time of 1 min and a cycle time of 5 min, the preparation being washed twice between doses. Phenoxybenzamine was then applied to the tissue and allowed to act for 30 min, at which time the vesicle was washed twice and the responses to the agonist again observed. An isomer or the racemate of propranolol or INPEA was then added to the bathing medium and allowed to act for 15 min after which the preparation was washed for 15 min and the responses to the agonist again determined.

α-Adrenoceptor protection experiments. A four point dose response curve for noradrenaline or phenylephrine was obtained using a drug contact time of 1 min and a cycle time of 5 min. Phentolamine, or an isomer or the racemate of propranolol or INPEA was added to the bathing medium and allowed to act for 5 min, followed by phenoxybenzamine which was allowed to act for 5 or 30 min. The preparation was washed for 15 min and the responses to the agonist again determined. The results were compared with those of control experiments where the procedure was identical except that no β-adrenoceptor blocking agent or phentolamine was applied to the tissue.

Drugs and solutions

The drugs used were; phenylephrine hydrochloride; methoxamine hydrochloride Vasoxine; (−)-noradrenaline bitartrate; phenoxybenzamine hydrochloride; phentolamine methanesulphonate Rogitine; (−)-, (+), and (±)-propranolol hydro-

chloride and (−)-, (+)-, and (±)-INPEA- (*N*-isopropyl-*p*-nitrophenylethanolamine hydrochloride). All doses are quoted as the salt.

The composition of the Krebs solution was; NaCl 6·92; KCl 0·35; $CaCl_2$ 0·28; $NaHCO_3$ 2·10; NaH_3PO_4 0·16; $MgSO_4.7H_2O$ 0·29 and glucose 2·00 g/litre of distilled water.

RESULTS

Rat blood pressure

Reversal of α-adrenoceptor blockade. The inhibition of the pressor responses of 0·31 to 10·0 μg of noradrenaline, produced by 2·5 mg/kg of phenoxybenzamine were partially reversed by (−)- and (±)-propranolol (1 mg/kg) or (−)- and (±)-INPEA (10 mg/kg). (−)-Propranolol or (−)-INPEA were only slightly more effective than the corresponding racemate. No reversal was observed when the α-adrenoceptor blockade was challenged with (+)-propranolol (1 mg/kg) or (+)-INPEA (10 mg/kg). The results are in Fig. 1. The control responses before and after phenoxybenzamine were pooled. Phenoxybenzamine, 2·5 mg/kg almost abolished (90 %) the pressor responses to the higher doses of noradrenaline, and propranolol was found to be more than 10 times more active than INPEA in its reversal of phenoxybenzamine-produced α-adrenoceptor blockade. The phenoxybenzamine-produced inhibition of the pressor responses to 1·25–20 μg of phenylephrine and 2·5–80 μg of methoxamine were not reversed by (−)- or (±)-propranolol or (−)- or (±)-INPEA.

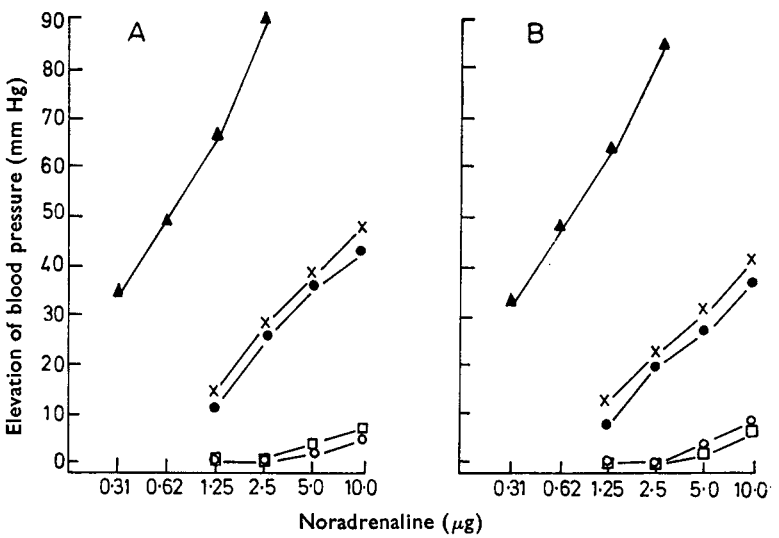

FIG. 1. The pressor responses of the rat blood pressure to noradrenaline in the propranolol series (A) and of the INPEA series (B) of experiments. ▲ — ▲ Control (noradrenaline alone); □ — □ Phenoxybenzamine; ○ — ○ Phenoxybenzamine with (+)-isomer; × — × Phenoxybenzamine with (−)-isomer; ● — ● Phenoxybenzamine with (±)-mixture. The doses were phenoxybenzamine 2·5 mg/kg; isomers and racemate of propranolol 1 mg/kg, and the isomers and racemate of INPEA, 10 mg/kg. Each point represents the mean of 6 determinations.

Before the administration of phenoxybenzamine, the resting blood pressure was within the range of 70–110 mm Hg and after the development of α-adrenoceptor blockade the blood pressure was lowered to 28–42 mm Hg where it stayed for the remainder of the experiment. In the presence of phenoxybenzamine, both the

isomers and racemates of propranolol and INPEA produced marked pressor effects, of 20–47 mm/Hg which were not well maintained, the blood pressure returning to the original level within 10 min.

β-Adrenoceptor blockade. The effects of the isomers and racemates of propranolol (1 mg/kg) and of INPEA (10 mg/kg) on the fall in blood pressure produced by 6·2–25 ng of isoprenaline are shown in Fig. 2. (−)-, and (±)-Propranolol or (−)-INPEA abolished and (±)-INPEA greatly reduced the response to isoprenaline but (+)-propranolol and (+)-INPEA had little effect upon the isoprenaline-produced fall in blood pressure.

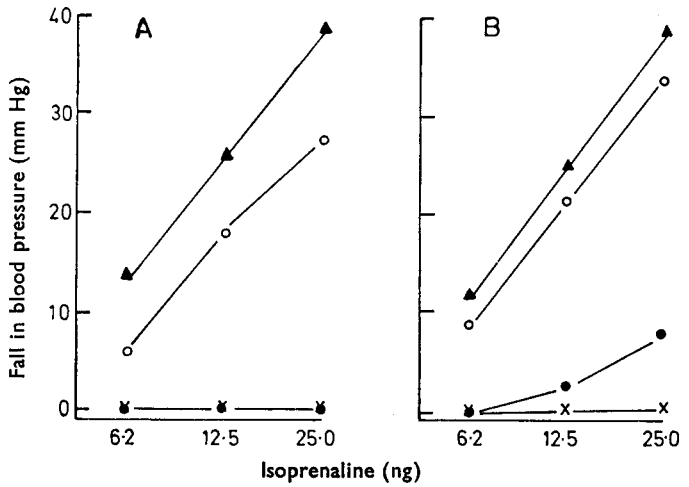

FIG. 2. The depressor responses of the rat blood pressure to isoprenaline in the propranolol series (A) and the INPEA series (B) of experiments. ▲ — ▲ Control (isoprenaline alone); ○ — ○ (+)-isomer; × — × (−)-isomer; ● — ● (±)-mixture. The doses were for the isomers and racemate of propranolol 1 mg/kg and for the isomers and racemate of INPEA 10 mg/kg. Each point represents the mean of 6 determinations.

Guinea-pig seminal vesicle preparation

Effect of β-adrenoceptor blocking agents on α-adrenoceptor blockade. Contractions of the seminal vesicle were obtained with 1·25–20 μg/ml of noradrenaline and 5·0–100 μg/ml of phenylephrine. There was a delay between the contract of the drug with the tissue and the tissue response which was most marked with the lower concentrations, lasting for as long as 25s. Phenoxybenzamine, 12·5 ng/ml, was allowed to act for 30 min and produced a marked reduction in the responses to noradrenaline and phenylephrine. The responses to the higher concentrations were reduced by more than 80%. The α-adrenoceptor blockade produced, persisted after repeated washings and challenge with agonist.

Neither isomer, nor the racemate of propranolol or INPEA in doses up to 100 μg/ml modified the responses of the phenoxybenzamine-treated seminal vesicles to the agonists. The isomers of propranolol or INPEA did not exhibit any intrinsic activity but in doses greater than 25 and 75 μg/ml respectively, they produced an inhibition of the response of the seminal vesicle to the agonist. This effect was readily reversed by washing the preparation with Krebs solution. Methoxamine was not used in these studies because in the concentrations of 2·5–10 μg/ml it produced persistent spontaneous activity.

α-Adrenoceptor protection experiments. Two contact times for phenoxybenzamine were employed, and at 5 min, 50 ng/ml of phenoxybenzamine produced a similar inhibition of the noradrenaline- or phenylephrine-produced contractions as did 12·5 ng/ml after 30 min. When the contact time of phenoxybenzamine was 5 min, the isomers or racemates of propranolol or INPEA, in concentrations of 25–100 μg/ml, or phentolamine, 0·1–2·0 μg/ml, applied to the tissue 5 min before the phenoxybenzamine, partially prevented the inhibition of the contractions of the seminal vesicle produced by phenylephrine (Fig. 3). The isomers were equi-effective in preventing the development of phenoxybenzamine α-adrenoceptor blockade and propranolol was no more effective than INPEA. However, when the contact time of phenoxybenzamine was 30 min, preparations pretreated with a β-adrenoceptor were not protected from the blocking action of phenoxybenzamine.

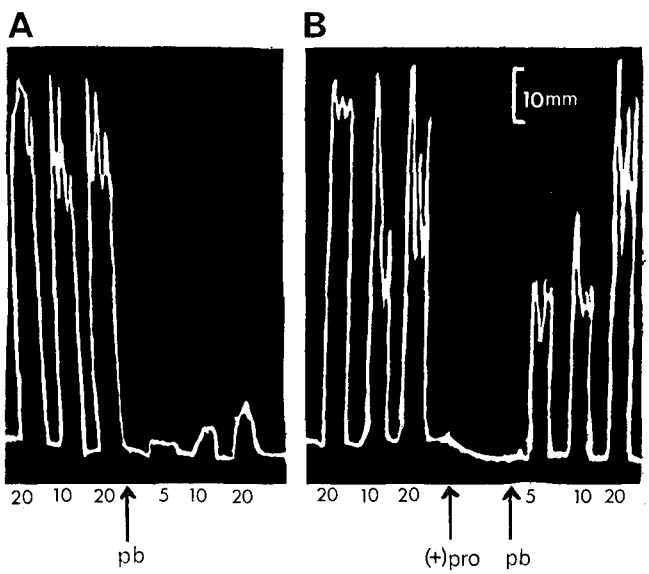

FIG. 3. Kymograph record of the responses of the guinea-pig seminal vesicle to noradrenaline, 5–20 μg/ml. Record A is a control showing the responses before and after phenoxybenzamine 50 ng/ml (pb). Record B shows the effect of (+)-propranolol 100 μg/ml (pro) administered 5 min before the phenoxybenzamine, on the phenoxybenzamine-produced inhibition of the noradrenaline-evoked responses. The contact time for phenoxybenzamine was 5 min.

DISCUSSION

In the urethane-anaesthetized rat, the inhibition of the pressor effects of noradrenaline produced by phenoxybenzamine were partially reversed by (−)- and (±)-propranolol and (−)- and (±)-INPEA. No reversal was observed with (+)-propranolol or (+)-INPEA. Phenoxybenzamine-produced inhibition of the pressor effects of phenylephrine—an amine with a marked α- and weak β-adrenoceptor activity—and methoxamine—an amine exhibiting only α-adrenoceptor activity—was not reversed by (−)- or (±)-propranolol or (−)- or (±)-INPEA. These observations are similar to those reported by Yamamura & Horita (1968) on the effects of (±)-propranolol on α-adrenoceptor blockade in the cat. Propranolol was more than 10 times more active than INPEA and in the doses employed, (−)- and (±)-propranolol and (−)- and (±)-INPEA abolished or greatly reduced the vasodepressor

effects of isoprenaline whilst the same dose of (+)-propranolol and (+)-INPEA exhibited very little β-adrenoceptor blocking activity (Howe & Shanks, 1966; Patil, 1968). That the phenoxybenzamine-produced α-adrenoceptor blockade is reversed by the β-adrenoceptor blocking agents only when the agonist is an amine with a significant β- as well as α- activity, and the isomer that exhibits little β-adrenoceptor blocking activity does not produce a reversal of α-adrenoceptor blockade, indicates that β-adrenoceptor blockade plays an important part in the reversal. The hypothesis that the inhibition of β-adrenoceptor activity results in the unmasking of residual α-adrenoceptor activity not blocked by the α-adrenoceptor blocking agent is therefore suggested by these observations. Both isomers and racemates of propranolol and of INPEA produced pressor responses in the presence of phenoxybenzamine. Similar observations have been reported for (±)-propranolol in the rat by Yamamoto & Sekiya (1969).

From the *in vitro* studies on the guinea-pig seminal vesicle preparation, it was found that prior administration of phentolamine or the isomers or racemates of propranolol or INPEA partially prevented the development of inhibition by phenoxybenzamine of the noradrenaline- and phenylephrine-produced contractions over 5 min. The isomers were equi-effective in preventing the development of α-adrenoceptor blockade and there was no difference in the effective concentrations of propranolol and INPEA. Patil & others (1968) observed similar activities of isomers of propranolol and INPEA in the vas deferens preparation in protecting α-adrenoceptors from dibenamine blockade. In the present experiments, when the duration of action of phenoxybenzamine was extended to 30 min, the administration of phentolamine or the isomers or racemates of propranolol or INPEA before or after the development of α-adrenoceptor blockade did not prevent the development of, or reverse the α-adrenoceptor blockade. The development of α-adrenoceptor blockade by phenoxybenzamine is complex. Initially the block is competitive and may be antagonized by catecholamines or competitive α-adrenoceptor blocking agents. When the block is complete, it is of a non-competitive nature and is unaffected by the presence of catecholamines or competitive α-adrenoceptor blocking agents (Nickerson & Gump, 1949; Furchgott, 1954). The inhibition of the development of phenoxybenzamine-produced α-adrenoceptor blockade seen in the seminal vesicle experiments cannot be mediated through the blockade of β-receptors, because the isomers are equi-effective in their protective activity and the seminal vesicle preparation exhibits only α-adrenoceptor activity (Guimaraes, 1969). These results support the hypothesis that the β-adrenoceptor blocking agents are capable of occupying and competing with phenoxybenzamine for the α-adrenoceptor. However, in view of the high concentrations required to produce this effect, the β-adrenoceptor blocking agents would appear to have a very weak activity at the α-receptor. Guimaraes (1969), using the guinea-pig seminal vesicle preparation, showed that (±)-pronethanol would protect the α-receptor from dibenamine blockade for a more than 20 min. Propranolol and INPEA did not afford protection of the α-receptors from phenoxybenzamine blockade when the contact time of the phenoxybenzamine exceeded 30 min. The differences in the duration of protection afforded by the β-adrenoceptor blocking agents from dibenamine and phenoxybenzamine α-adrenoceptor blockade may be explained by the fact that dibenamine has a slow onset of action (Nickerson & Goodman, 1947) and therefore a slower development of non-competitive α-adrenoceptor blockade. The inhibition of the responses of the seminal

vesicles to agonists produced by propranolol and INPEA is most probably a local anaesthetic action (see Davis, 1970).

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