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Antagonism by propranolol of the inhibitory effect of phenoxybenzamine on noradrenaline uptake *in vivo*

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The reduction of noradrenaline stores and [^{3}H]noradrenaline concentration in the heart of mice and rats induced by phenoxybenzamine-treatment, alone or in combination with cold-stress, was prevented by propranolol. Propranolol also antagonized a similar effect induced by phentolamine but not that induced by other noradrenaline uptake inhibitors, such as desipramine, cocaine, guanethidine and reserpine. Analysis of the time-course of antagonism by propranolol indicates that it was evident only when the β-adrenoceptor blocking agent remained in the body. The inhibitory effect of phenoxybenzamine on noradrenaline stores reappeared when propranolol was excreted. Propranolol alone did not change cardiac noradrenaline stores or [^{3}H]noradrenaline. It is concluded that the restoration of reflexly increased adrenergic discharge to normal, because of unmasking of spare α-adrenoceptors resulting from β-adrenoceptor blockade by propranolol rather than competition for binding at the active site of phenoxybenzamine, is responsible for the observed antagonism.

In addition to blockade of α-adrenoceptors, phenoxybenzamine (PBZ) has been shown to exert various effects on the adrenergic effector system. It inhibits neuronal uptake of noradrenaline (Hertting, Axelrod & Whitby, 1961; Stafford, 1963; Iversen, 1965; Iversen & Langer, 1969), decreases noradrenaline stores (Schapiro, 1958; Farrant, Harvey & Pennefather, 1964; Reid, Volicer & Brodie, 1969), causes an overflow of noradrenaline during sympathetic nerve stimulation (Brown & Gillespie, 1957; Kirpekar & Cervoni, 1963; Thoenen, Huerlimann & Haefely, 1964; Boullin, Costa & Brodie, 1967; Kirpekar & Wakade, 1970), increases its synthesis (Dairman, Gordon & others, 1968; Reid & others, 1969) and inhibits its extraneuronal uptake as well as its metabolism (Eisenfeld, Axelrod & Krakoff, 1967; Eisenfeld, Landsberg & Axelrod, 1967; Lightman & Iversen, 1969). PBZ also protects adrenergic neurons from the blocking effect of guanethidine (Thoenen, Huerlimann & Haefely, 1966; Matsumoto, 1966; Kirpekar, Wakade & others, 1969).

β-Adrenoceptor blocking drugs, including propranolol, have been shown to reverse α-adrenoceptor blockade induced by PBZ (Hull, Eltherington & Horita, 1960; Moreira & Osswald, 1965; Tuttle, 1965; Gulati, Gokhale & Udwadia, 1965; Eble & Rudzik, 1966; Garrett, Malafaya-Baptista & Osswald, 1966; Olivares, Smith & Aronow, 1967; Yamamura & Horita, 1968, 1969; Smith & Nash, 1969) and also to protect the α-adrenoceptor from irreversible binding with PBZ (Kohli & Ling, 1967; Patil, Tye & others, 1968; Mazurkiewicz-Kwilecki, 1970). The present paper describes other aspects of this antagonism between propranolol and PBZ. It will be shown that the inhibition of noradrenaline accumulation, as well as its reduction

in cardiac stores in mice and rats, caused by PBZ is prevented by the administration of propranolol.

METHODS

Long Evans rats of either sex weighing 180–250 g and NIH mice weighing 22–28 g were used.

Uptake of [^{3}H]noradrenaline

To each mouse 2 μCi (0·24 μg) of (±)-[1-^{3}H]noradrenaline (^{3}H-NA) in 0·2 ml was given intravenously in the tail vein, while for the rat, 25 μCi (3 μg)/kg of ^{3}H-NA was injected. After various intervals the animals were killed, the hearts rapidly excised and homogenized in a motor driven glass homogenizer with ice-cold 0·4N $HClO_4$. An aliquot (0·2 ml) of the supernatant was counted for its total radioactivity in a liquid scintillation spectrometer. The radioactivity was expressed as d min^{-1} g^{-1} wet tissue in the experiments using rats or as d min^{-1} per heart in the experiments using mice.

Assay of ^{3}H-NA

To an aliquot (2 ml) of the supernatant of the crude homogenate 0·2 g Al_2O_3 was added and the pH adjusted to about 8 by adding 90 mg $NaHCO_3$ and 0·5 ml 0·2M tris buffer. The catecholamines adsorbed onto Al_2O_3 were then eluted with 2 ml 0·1N HCl and the radioactivity contained in 0·2 ml of the eluate was counted. Since the amount of deaminated catechols in the heart is negligible (Eisenfeld & others, 1967) the count was taken to represent unchanged ^{3}H-NA.

Assay of endogenous noradrenaline

Eluates obtained from Al_2O_3 as described above were assayed for endogenous noradrenaline using a fluorospectrophotometric method (Chang, 1964).

Cold-stress

Rats, one each in a small cage, were put in a cold room at 6° for 60 min. After the cold-exposure, animals were killed immediately for assay of noradrenaline, total radioactivity and ^{3}H-NA.

The drugs used were metanephrine hydrochloride, phenoxybenzamine hydrochloride, propranolol hydrochloride, cocaine hydrochloride, desipramine hydrochloride, guanethidine sulphate, phentolamine mesylate, reserpine and (±)-[1-^{3}H]-noradrenaline (1400 mCi/mmol). The doses of the drugs except those of reserpine and noradrenaline refer to the salts.

RESULTS

Uptake and disappearance of ^{3}H-NA in the heart of mice

After intravenous injection, ^{3}H-NA was rapidly incorporated into the heart and then declined with two exponential components as reported previously by Montanari, Costa & others (1963). The half-life of the second exponential phase was about 7 h. The unchanged ^{3}H-NA appeared to be about 90% of the total radioactivity.

Pretreatment of the animals with metanephrine (25 mg/kg, s.c.), a potent inhibitor

Table 1. *Effects on the concentration of ^{3}H-NA in the heart of mice.* Each mouse received intravenously 2 μCi ^{3}H-NA and was killed 10 min or 5 h after injection. PBZ was given 2 h before, and propranolol or metanephrine 1 h before, ^{3}H-NA. The experiments were at room-temperature (18–25°).

Treatment	Dose (mg/kg, s.c.)	Total radioactivity (d min^{-1} per heart ± s.e.) 10 min	5 h
Control	—	24 900 ± 1350 (n = 31)	8690 ± 528 (n = 28)
Metanephrine	25	25 700 ± 4230 (n = 3)	8340 ± 1040 (n = 3)
PBZ	10	17 300 ± 406* (n = 13)	3760 ± 428* (n = 14)
Propranolol	5	24 100 ± 1030 (n = 25)	9920 ± 560 (n = 21)
PBZ + propranolol	10, 5	21 500 ± 689** (n = 7)	5670 ± 744** (n = 7)

* $P<0.05$ *vs* control.
** $P<0.05$ *vs* PBZ alone.

Table 2. *Effects of propranolol on the inhibition of noradrenaline uptake in the heart of mice caused by various agents.* All inhibitory drugs were given subcutaneously 2 h before ^{3}H-NA injection. Propranolol (5 mg/kg, s.c.) was administered 1 h after each inhibitory drug. Mice were killed at 5 h after ^{3}H-NA. The experiments were at room-temperature (18–25°).

Treatment	Dose (mg/kg)	Total radioactivity (d min^{-1} per heart ± s.e.) without propranolol	with propranolol
Control	—	8690 ± 528 (n = 28)	9920 ± 560 (n = 21)
Guanethidine	10	1920 ± 95 (n = 3)	2024 ± 111 (n = 5)
Reserpine	1	966 ± 187 (n = 4)	838 ± 82 (n = 4)
Desipramine	5	1820 ± 250 (n = 3)	1350 ± 258 (n = 3)
Cocaine	15	5160 ± 358 (n = 3)	5750 ± 510 (n = 5)
Phentolamine	15	5680 ± 484 (n = 10)	8870 ± 774* (n = 8)
PBZ	10	3760 ± 428 (n = 14)	5670 ± 744* (n = 6)

* $P<0.05$ *vs* without propranolol.

of extraneuronal noradrenaline uptake but without appreciable effect on the intraneuronal uptake (Iversen 1965; Eisenfeld & others, 1967), appeared to have no appreciable effect on the total radioactivity either when the animal was killed 10 min or 5 h after administration of ^{3}H-NA (Table 1). When compared with the marked effect caused by other inhibitors of neuronal uptake, such as guanethidine, desipramine and cocaine (Table 2), it may be inferred that the extraneuronal uptake of ^{3}H-NA contributed to a negligible extent to the observed total radioactivity in the present experiments.

Effect of PBZ on ^{3}H-NA concentration in mice

When mice were pretreated with a single dose of PBZ (10 mg/kg, s.c.) 2 h before ^{3}H-NA, the total radioactivity in the heart was about 30% lower than that in the control animals when the mice were killed 10 min after ^{3}H-NA injection, and about

Table 3. *Prevention by propranolol of the reduction of ^{3}H-NA caused by PBZ in the rat heart.* Rats, placed in a warm environment (27–30°), were treated with PBZ (10 mg/kg, i.v.) and /or propranolol (10 mg/kg, i.v.) 10 min before injection of ^{3}H-NA (25 μCi/kg, i.v.) and killed 60 min later.

	Total radioactivity (d min^{-1} g^{-1} ± s.e.)		^{3}H-NA (% of total radioactivity)	
Rats	without propranolol	with propranolol	without propranolol	with propranolol
Control	121 000 ± 5230 (n = 6)	119 000 ± 1470 (n = 4)	95 ± 1·8	92 ± 3·1
PBZ-treated ..	46 200 ± 7700* (n = 3)	120 000 ± 10 790** (n = 4)	93 ± 3·7	92 ± 3·4

* $P<0·05$ *vs* control.
** $P<0·05$ *vs* PBZ only.

60% lower when the animals were killed at 5 h (Table 1). This may indicate that, in addition to competitive inhibition of the neuronal uptake (Iversen & Langer, 1969), PBZ also increases the reflex sympathetic discharge and turnover of noradrenaline due to blockade of α-adrenoceptors (Reid & others, 1969). Inhibition by PBZ of extraneuronal noradrenaline uptake (Eisenfeld & others, 1967; Iversen & Langer, 1969) was probably not involved since little extraneuronal binding of the amine was involved under the present experimental conditions.

Antagonism by propranolol in mice

The inhibitory effect of PBZ on ^{3}H-HA concentrations was significantly reduced when the mouse was treated with propranolol (5 mg/kg, s.c.) 1 h after PBZ administration. The data in Table 1 show that the total radioactivity in the heart of the PBZ-treated mice was partially restored. Propranolol, when given alone, did not appreciably affect the ^{3}H-NA concentrations, or noradrenaline stores, of hearts either in the mouse (Table 1) or in the rat (Table 3).

Whether the inhibitory effect on ^{3}H-NA uptake by other drugs might also be prevented by propranolol was tested. The results illustrated in Table 2 indicate that none of the effects of reserpine, guanethidine, cocaine or desipramine was appreciably reduced by propranolol, whereas the inhibition caused by phentolamine was reversed in a similar manner to that of PBZ.

Antagonism by propranolol in rats

The antagonism between propranolol and PBZ appeared to be more evident in this species. When ^{3}H-NA was administered to rats 10 min after intravenous injection of PBZ (10 mg/kg), the radioactivity remaining in the heart 60 min after injection of ^{3}H-NA was about 60% lower than in the controls. Simultaneous treatment with propranolol (10 mg/kg, i.v.) completely antagonized this effect of PBZ (Table 3). No appreciable change in the proportion of ^{3}H-NA to total radioactivity in the heart was observed in PBZ- or propranolol-treated animals, or in animals given both drugs (Table 3). This indicates that the effect is not simply due to increased retention of metabolites.

Time-course of the antagonism by propranolol

To ascertain the mechanism involved in the antagonism by propranolol of PBZ, propranolol (10 mg/kg, i.p.) was administered to the rat at various times after pre-

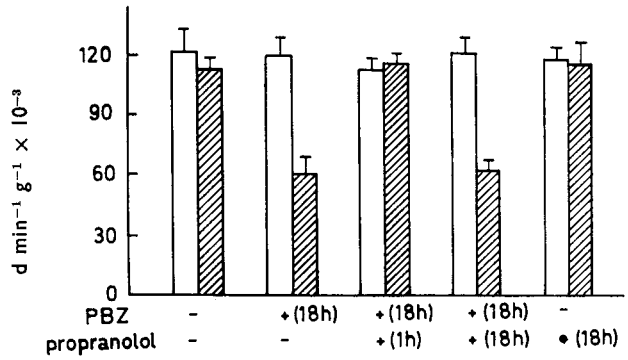

FIG. 1. Time-course of the antagonism by propranolol of the reduction of ^{3}H-NA concn in the rat heart induced by PBZ treatment and cold-stress. PBZ (10 mg/kg, i.p.) and propranolol (10 mg/kg, i.p.) were given as indicated in the bottom of the figure at the time shown in parentheses before injection of ^{3}H-NA. The rats were killed 60 min after ^{3}H-NA. Open columns show the mean d min^{-1} g^{-1} heart ± s.e. of control rats (n = 4–5) kept in 30° environment throughout the experiment; shaded columns show the values of rats kept in 6° environment for the last 60 min before death.

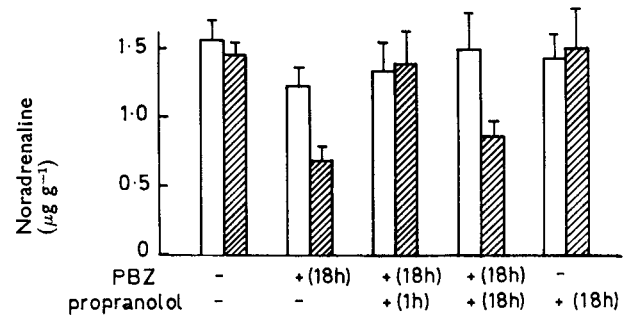

FIG. 2. Antagonism by propranolol of the PBZ-induced depletion of noradrenaline stores in the rat heart. Experimental conditions were the same as those shown in the legend to Fig. 1. The data are mean ± s.e. (n = 4–9).

treatment with PBZ. It was found that PBZ (10 mg/kg), administered intraperitoneally to animals kept in a warm environment (30°), did not cause any change in the amount of ^{3}H-NA in the heart. However, when the animals were placed in a refrigerator (6°) for 1 h, a marked reduction in the ^{3}H-NA concentration in the heart was observed. This effect of PBZ persisted, being still evident 18 h after PBZ administration. Advantage of this persistent effect was taken to test whether the antagonism by propranolol involved competition for binding at the active site, or that the pharmacological effect of propranolol *per se* was responsible for the antagonism. The experiments shown in Fig. 1 reveal that propranolol was effective when given shortly before the injection of ^{3}H-NA even when PBZ had been administered 18 h previously. In contrast, propranolol was ineffective when given simultaneously with PBZ.

Effect on noradrenaline stores

PBZ has been shown to increase the turnover rate of noradrenaline and decrease the amount of it in stores (Schapiro, 1958; Farrant & others, 1964; Reid & others,

1969). We found that this effect of PBZ in the heart could be negated when the rat was placed in a warm environment (30°), but was markedly enhanced when placed in a cold environment (6°, for 1 h). As illustrated in Fig. 2, this depleting effect of PBZ on heart noradrenaline stores was completely antagonized by propranolol provided it was administered shortly before the cold-stress.

DISCUSSION

The results of the present experiments demonstrate that the reduction of ^{3}H-NA accumulation, as well as that of cardiac noradrenaline stores, induced by PBZ treatment, was prevented by propranolol. The time-course of this antagonism indicates that it is not due to prevention, by propranolol, of the binding of PBZ with the active site. Rather, it is likely that a pharmacological action of the propranolol *per se* is responsible for the effect.

PBZ may cause reduction of noradrenaline cardiac stores, or of accumulation of ^{3}H-NA, *in vivo* either by inhibiting the uptake or by reflexly increasing cardiac adrenergic discharge resulting from α-adrenoceptor blockade. The findings that propranolol also antagonized the inhibitory effect on ^{3}H-NA accumulation by phentolamine but none of those induced by uptake inhibitors, such as desipramine, cocaine, guanethidine or reserpine, suggest that it is the reflexly increased adrenergic discharge which is antagonized by propranolol since both PBZ and phentolamine are inhibitors of α-adrenoceptors. Indeed, our recent experiments show that, in rats, the reduction of noradrenaline stores or decreased accumulation of ^{3}H-NA in the heart *in vivo* by PBZ is not due to inhibition of noradrenaline uptake but rather to increase of turnover rate (Chang & Lee, in preparation). It may be that the reversal by propranolol of PBZ-induced α-adrenoceptor blockade (Olivares & others, 1967; Yamamura & Horita, 1968, 1969; Smith & Nash, 1969; Mazurkiewicz-Kwilecki, 1970) restores the reflexly increased adrenergic discharge to normal and consequently removes the cause of decline of noradrenaline stores.

Two hypotheses have been proposed to explain the reversal by propranolol of PBZ-induced α-adrenoceptor blockade. Olivares & others (1967), observing that the antagonistic effect of propranolol persisted longer than its β-adrenoceptor blocking activity, concluded that the antagonism was due to displacement of PBZ from the receptor. Yamamura & Horita (1968, 1969), however, were not able to confirm this. Smith & Nash (1969) and Yamamura & Horita (1968, 1969) concluded, on the basis that the reversal occurred only with (−)-propranolol and that the reversal was observed only when α-adrenoceptor agonists which also had β-agonist properties were used, that the reversal was due to unmasking of unblocked 'spare' α-receptors resulting from β-receptor inhibition by propranolol. Our experiment on the time-course of the antagonism of the effect of PBZ on noradrenaline storage seems to support this conclusion.

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