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Effect of Timolol on Hydralazine-Induced Increase in Plasma Renin Activity in Spontaneously Hypertensive and Normotensive Rats (39567)

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Timolol is a β-adrenergic-blocking agent which has been found to be relatively free of direct cardiac-depressant activity in animals (1, 2). Scriabine *et al.* (3) reported that timolol potentiated the antihypertensive response to hydralazine in spontaneously hypertensive rats (SHR). We studied the effects of timolol and propranolol on plasma renin activity (PRA) in SHR and normotensive rats. Antagonism of hydralazine-induced increases in PRA by single or multiple oral doses of timolol and propranolol was studied in SHR, normotensive Wistar (NWR), and normotensive Sprague-Dawley (NSDR) rats. We found considerable differences in the responses of various strains of rats to these β-adrenergic-blocking agents.

Methods. SHR of the Wistar-Okamoto strain were obtained from Charles River-Lakeview (Wilmington, Mass.). All rats were healthy females weighing 200 to 225 g. SHR were approximately 15-18 weeks of age while NWR and NSDR were approximately 12-15 weeks of age. All rats were allowed free access to food and water for at least 1 week prior to study and were fasted for 16 hr prior to oral drug administration. Drugs were dissolved in distilled water and administered by gavage (1 ml/kg body weight). Doses of all drugs were calculated in terms of the base. All rats were randomly divided into experimental groups.

All rats were sacrificed by decapitation. The initial 4 to 5 ml of trunk blood was collected in iced cups containing EDTA. PRA was estimated by radioimmunoassay for angiotensin I (A1) (Schwarz/Mann kit), and expressed as nanograms of A1 formed per milliliter of plasma per hour of incubation at 37°. All plasma samples from each study were assayed for PRA simultaneously to reduce potential interassay variability. The experimental protocols were as follows.

Protocol No. 1. Groups of SHR were given single oral doses of (1) hydralazine,[2] 1 mg/kg; (2) timolol, 2 mg/kg; or (3) timolol, 2 mg/kg, followed by hydralazine, 1 mg/kg, 15 min later. A fourth group received 1 ml/kg of distilled water as a placebo. PRA was determined from plasma samples obtained 1 hr after administration of the drugs.

Protocol No. 2. Changes in PRA were determined in 15 groups of SHR after single oral doses of timolol, 2 mg/kg; propranolol,[3] 10 mg/kg; or hydralazine, 4 mg/kg. Four rats were used in each group; five groups were used per treatment. The animals were sacrificed and blood samples for PRA assay were obtained at 0, 0.5, 1, 2, and 6 hr after drug administration.

Protocol No. 3. Dose-response studies with single oral doses of timolol or propranolol were conducted in SHR, NWR, and NSDR. Timolol was given at 0.5, 2, and 8 mg/kg, and propranolol at 2.5, 10, and 40 mg/kg. Four animals were used at each dose level of each drug. One group (four animals) of each strain of rats received distilled water as a placebo. The animals were sacrificed and blood samples were obtained 1 hr after drug administration. Geometric means ± SE for PRA were calculated for each group.

Protocol No. 4. Groups of 9 or 10 SHR, NWR, and NSDR were treated orally with hydralazine, 4 mg/kg; timolol, 2 mg/kg; or hydralazine, 4 mg/kg, + timolol, 2 mg/kg, once daily for 4 consecutive days. In the last group, timolol was given 15 min prior to hydralazine. A fourth group received distilled water as a placebo. The animals were

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[2] Hydralazine was supplied by Dr. A. J. Plummer, Ciba-Geigy Corp., Summit, N. J.

[3] Propranolol was purchased from Aldrich Chemical Co., Milwaukee, Wis.

sacrificed and the blood samples were obtained at 1 hr after drug administration on the fourth day of treatment.

Protocol No. 5. Groups of 10 NSDR were treated orally with hydralazine, 4 mg/kg/day; timolol, 10 mg/kg/day; propranolol, 10 mg/kg/day; timolol, 10 mg/kg/day + hydralazine, 4 mg/kg/day; or propranolol, 10 mg/kg/day, + hydralazine, 4 mg/kg/day, for 4 consecutive days. β-Adrenergic-blocking agents were given 15 min prior to hydralazine in the last two groups. Two groups received distilled water as a placebo. All blood samples were obtained at 1 hr after drug administration and geometric means ± SE for PRA were calculated for each group.

Treatment-related changes in PRA were analyzed by a one-way analysis of variance procedure for each protocol.

Results. Protocol No. 1. PRA in 15 placebo-treated SHR was 2.7 ± 0.2 ng of A1/ml/hr (Table I).

In 16 SHR given hydralazine, PRA was 3.9 ± 0.4 ng of A1/ml/hr, which is significantly higher than in the placebo-treated group ($P < 0.02$). PRA in 16 SHR given timolol was 1.9 ± 0.2 ng of A1/ml/hr and was significantly lower ($P < 0.05$) than in the placebo group. In 15 SHR given timolol + hydralazine, PRA was not significantly higher than in placebo-treated SHR (3.2 ± 0.5 ng of A1/ml/hr).

Protocol No. 2. In placebo-treated SHR, PRA immediately after treatment was 1.7 ± 0.4 ng of A1/ml/hr. Timolol or propranolol produced equivalent reductions in PRA ($P < 0.05$) after 0.5 to 2 hr (Fig. 1).

Protocol No. 3. Peripheral PRA responses to graded, single oral doses of timolol or propranolol in SHR, NWR, and NSDR are shown in Fig. 2. In SHR (Fig. 2A), there was a negative, linear correlation between doses of β-adrenergic-blocking agents and PRA, and the slopes of regression lines were identical (see Fig. 2).

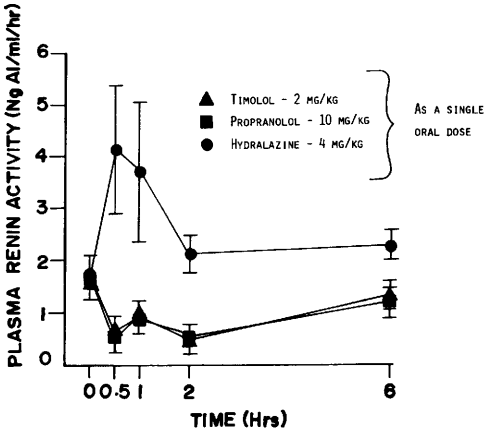

FIG. 1. Duration of action of timolol, propranolol, and hydralazine on PRA in spontaneously hypertensive rats. Each value represents the geometric mean ± SE for a group of four rats.

In NWR, timolol and propranolol at doses up to 10 mg/kg p.o. had no significant effect on PRA (Fig. 2B). Only at 40 mg/kg p.o., did propranolol lower PRA in NWR.

At the doses used, neither of the two β-adrenergic-blocking drugs had any dose-dependent effect on PRA in NSDR (Fig. 2C), although the PRA in rats treated with timolol was consistently lower than in the placebo-treated group.

Protocol No. 4. The results are summarized in Table II. There were no significant differences in PRA of placebo-treated rats from all three strains. These results support the findings of Sen *et al.* (4) that peripheral PRA in SHR is essentially the same as in NWR.

TABLE I. EFFECTS OF HYDRALAZINE AND TIMOLOL, BY SINGLE ORAL DOSES ON PRA IN SHR.

Group no.	Treatment	Dose (mg/kg, p.o.)	Number of rats per group	Plasma renin activity (PRA)[a,b] (ng of A1/ml/hr ± SE)
1	Distilled water, 1 ml/kg	–	15	2.7 ± 0.2
2	Hydralazine	1	16	3.9 ± 0.4
3	Timolol	2	16	1.9 ± 0.2
4	Timolol	2		
	+	+	15	3.2 ± 0.5
	Hydralazine	1		

[a] PRA values are expressed as the geometric mean ± SE.
[b] Blood samples for PRA assay were obtained 1 hr after drug administration.

Hydralazine produced equivalent increases in PRA in all three rat strains. Timolol significantly lowered peripheral PRA in SHR ($P < 0.001$) and in NWR ($P < 0.001$), but not in NSDR. The combination of timolol + hydralazine appeared to reduce PRA in SHR, but the effect did not reach a statistically significant level. In NSDR, PRA was significantly increased after timolol + hydralazine ($P < 0.001$). The comparison between rat strains revealed that after combined treatment with timolol + hydralazine, PRA in SHR was significantly lower than in NWR and NSDR, and PRA in NWR was significantly lower than in NSDR (both at $P < 0.05$).

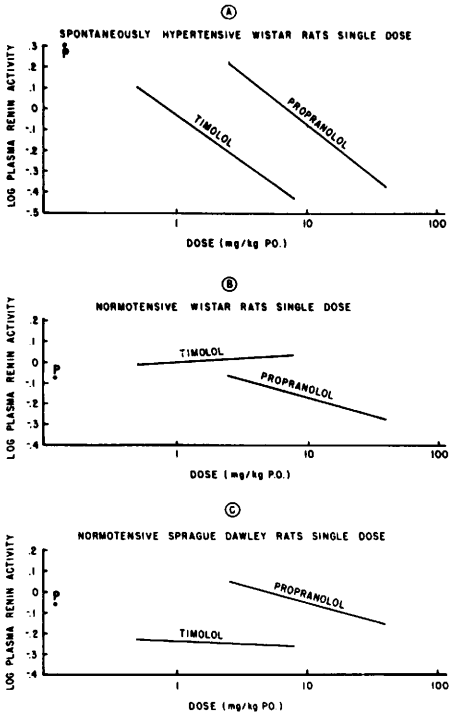

FIG. 2. Dose-response regression lines for the effects of timolol and propranolol on PRA in spontaneously hypertensive and normotensive rats. Timolol was given at 0.5, 2, and 8 mg/kg, p.o., and propranolol at 2.5, 10, and 40 mg/kg, p.o., to groups of four rats 1 hr before obtaining blood samples for PRA assay. Each value represents the geometric mean (expressed as log PRA) ± SE for a group of four rats. $\overset{P}{\bullet}$ Represents PRA in a group of placebo-treated rats of each strain. Slopes: (A) Timolol = −0.4455; propranolol = −0.4934. (B) Timolol = −0.0418; propranolol = −0.1840. (C) Timolol = −0.0206; propranolol = −0.1695.

Protocol No. 5. Two experiments were performed with this protocol in NSDR (Table III). PRA in two placebo groups was not statistically different. Likewise, PRA in the two hydralazine-treated groups was equally elevated.

Timolol or propranolol at 10 mg/kg/day significantly lowered PRA ($P < 0.05$ and $P < 0.01$, respectively). Timolol, 10 mg/kg/day, antagonized but did not prevent the hydralazine-induced elevation of PRA, while propranolol at the same dose prevented the hydralazine-induced elevation of PRA in NSDR.

Discussion. Peripheral PRA in the four groups of placebo-treated SHR was statistically equivalent and probably represents a range of normal PRA in active SHR subjected to daily handling and normal diet. Data from these rats also indicate that normal PRA in 200-g SHR is not significantly different from PRA in other strains of normotensive rats as was reported by Sen *et al.* (4).

TABLE II. EFFECTS OF HYDRALAZINE AND TIMOLOL (DAILY FOR 4 DAYS) ON PLASMA RENIN ACTIVITY IN VARIOUS STRAINS OF RATS.[a]

Strain of rats	Plasma renin activity (ng of Ang 1/ml/hr)			
	Distilled H_2O (1 ml/kg/day, p.o.)	Hydralazine (4 mg/kg/day, p.o.)	Timolol (2 mg/kg/day, p.o.)	Timolol + hydralazine
SH	2.0 ± 0.4	7.0 ± 2.0	0.5 ± 0.1	0.9 ± 0.3
NW	2.0 ± 0.2	8.7 ± 1.0	0.8 ± 0.1	2.0 ± 0.3
NSD	2.5 ± 0.3	9.0 ± 1.0	2.3 ± 0.2	4.8 ± 0.4

[a] Geometric means for 9 to 10 rats per group ± SE.

TABLE III. EFFECTS OF HYDRALAZINE AND β-ADRENERGIC-BLOCKING DRUGS (DAILY FOR 4 DAYS) ON PLASMA RENIN ACTIVITY (PRA) IN NSD RATS.[a]

Treatment	Dose (mg/kg/day, p.o.)	PRA[b] (ng of Ang 1/ml/hr) Expt. 1	Expt. 2
Distilled water (1 ml/kg/day)	–	2.5 ± 0.3	2.1 ± 0.2
Hydralazine	4	7.6 ± 0.9	7.7 ± 1.5
Timolol	10	1.6 ± 0.3	
Timolol + hydralazine	10 4	5.7 ± 0.7	
Propranolol	10		1.3 ± 0.1
Propranolol + hydralazine	10 4		2.4 ± 0.3

[a] Geometric means for 10 rats per group ± SE.
[b] PRA was determined at 1 hr after treatment on the fourth day.

Decapitation can be used as a reliable means of collecting rat blood for PRA assays, provided only the initial 4–5 ml of trunk blood are collected (5). Blood samples collected from rats under ether, halothane or barbiturate anesthesia are characterized by markedly elevated PRA (4–6).

According to Scriabine *et al.* (3), the antihypertensive effect of single oral doses of hydralazine in SHR is potentiated by timolol. Potentiation of the antihypertensive effects of hydralazine has been reported also for propranolol in SHR (7). Hydralazine-induced peripheral vasodilation probably leads to a reflex increase in PRA via secretion of adrenal catecholamines and/or via increased sympathetic nerve activity to renal juxtaglomerular cells (8, 9). By antagonizing the hydralazine-induced increase in PRA, timolol probably prevents subsequent formation of angiotensin II. After timolol, the hydralazine-induced relaxation of peripheral arteriolar smooth muscle was not opposed by circulating angiotensin II and, thus, the antihypertensive response to hydralazine was enhanced. Timolol, 2 mg/kg, lowered basal PRA in SHR in our studies, but did not reduce resting arterial pressure, as reported by Scriabine *et al.* (3). These observations indicate that generation of angiotensin is not required for maintenance of hypertension in mature SHR, but that the reflex responses to hydralazine-induced hypotension in SHR are angiotensin-dependent, just as in the NWR and NSDR.

Our experiments indicate that significant interstrain differences exist with regard to control of renin secretion in mature rats. In SHR, single oral doses of timolol and propranolol produce dose-dependent decreases of resting PRA (Fig. 2). The relative potency of timolol as a PRA-lowering agent in SHR was 7.7 times that of propranolol (95% confidence limits, 2.7 and 26.9 g = 0.1975). At the same doses, timolol did not reduce resting PRA in NWR, and propranolol was effective only at 40 mg/kg p.o. In NSDR, propranolol did not lower PRA even at 40 mg/kg p.o. and, at doses tested, timolol produced no dose-dependent effect on PRA. Because the regression lines for the effects of the two β-adrenergic-blocking agents on PRA in NWR and NSDR are invariant, the g-values are exceedingly high (g = 3.4019 and 2.0713, respectively). This suggests that NWR and NSDR are relatively insensitive models for the evaluation of the effects of β-adrenergic-blocking drugs on PRA.

Striking interstrain differences in effects on PRA were also seen when hydralazine + timolol were given daily for 4 days (Table II). Resting PRA and hydralazine-induced increases of PRA were equivalent in all strains. There were significant interstrain differences with regard to the effect of timolol on resting PRA and prevention of hydralazine-induced elevations of PRA. In SHR and NWR, timolol reduced resting PRA and also prevented the hydralazine-induced increase in PRA. In NSDR, timolol neither lowered resting PRA nor totally prevented the hydralazine-induced increase in PRA.

Reduction of resting PRA in NSDR by timolol and propranolol at 10 mg/kg/day for 4 days (but not by 2 mg/kg/day) suggests dose-dependent variability among rat strains with respect to renin suppression by these β-adrenergic-blocking agents.

Results of these experiments indicate that timolol and propranolol suppress resting PRA and prevent the hydralazine-induced increase in PRA most effectively in SHR, as compared with NWR or NSDR. Timolol is more potent than propranolol in suppressing PRA in SHR.

Summary. The effects of two β-adrenergic receptor-blocking agents, timolol and

propranolol, on resting PRA and on the hydralazine-induced increase in PRA were studied in SHR and in two strains of normotensive rats [Wistar (NWR) and Sprague-Dawley (NSDR)]. With single oral doses, these β-adrenergic-blocking drugs reduced PRA in a dose-dependent manner in SHR but not in NWR or NSDR. After four daily doses, timolol antagonized the hydralazine-induced increase in PRA in SHR and lowered PRA in NWR. Timolol lowered PRA in NSDR only at 10 mg/kg/day p.o. for 4 days. It was concluded that SHR are more sensitive than normotensive rats to the PRA-lowering effects of β-adrenergic-blocking drugs.

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