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Potentiation of the Antihypertensive Action of Hydralazine by Timolol in Spontaneously Hypertensive Rats (38136)

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According to clinical reports, β-adrenergic blocking agents are useful in treatment of hypertension, particularly in combination with peripheral vasodilator drugs such as hydralazine, dihydralazine or minoxidil and with diuretics (1–6). The combination therapy usually leads to satisfactory control of arterial pressure without postural hypotension, tachycardia or impairment of renal function.

Previous animal studies did not support the combined use of β-adrenergic blocking and peripheral vasodilator drugs in hypertension. Brunner *et al.* (7) found that propranolol antagonized the antihypertensive effect of hydralazine in renal hypertensive rats and according to Dahr *et al.* (8), practolol reduced the hypotensive effect of hydralazine in anesthetized dogs. We found, however, that timolol, a β-adrenergic blocking agent with the structure presented below (9–11), potentiated the antihypertensive effect of hydralazine in spontaneously hypertensive (SH) rats.

Methods. Spontaneously hypertensive (SH) male rats of the Wistar-Okamoto strain were purchased from Purina Farms (Vincentown, New Jersey). Their body weight ranged from 300–350 g and their age from 30–40 weeks. The aortic blood pressure was recorded directly through a catheter which was chronically implanted through the caudal artery. The animals were allowed free access to food and water. The blood pressure was recorded continuously through Statham P23Gb pressure transducers on a Honeywell Model 906C Visicorder. Heart rate was calculated from blood pressure tracings. All drugs were given by gavage. In studies involving treatments with 2 drugs, timolol or propranolol were administered 5 min prior to hydralazine. Four to eight rats were used with each dose of each drug or with each combination. The doses of all drugs were calculated in terms of base. Propranolol hydrochloride (racemic mixture) was purchased from Aldrich Chemical Co., Milwaukee, Wis. Hydralazine hydrochloride was supplied by Dr. A. J. Plummer as a courtesy of Ciba-Geigy Corporation.

The first series of experiments was largely exploratory without any specific experimental design. In the second series of experiments, a 6 × 6 Latin Square design was used. Rows and columns were represented by animals and periods of testing respectively. The animals were treated orally with saline; timolol, 0.5 mg/kg; hydralazine, 0.25 or 1.0 mg/kg; or combinations of timolol + hydralazine (Table I) every 48 hr to remove any possibility of carry-over effect. The duration of the study was, therefore, 12 days. In accordance with the Latin Square randomization scheme, each of the six treatments was repeated on each of the 6 animals. The maximal recorded fall in the arterial blood pressure within 24 hr after treatment was measured and was analyzed by the analysis of variance procedure associated with the Latin Square design (12). Duncan's multiple range test was used for the intercomparison of the treatments (13).

Results. Exploratory Experiments. The control mean arterial pressure of SH rats varied from 160–180 mm Hg. Folowing saline, 2 ml/kg orally, arterial pressure remained stable for a 24-hr period of observation. Timolol at 0.125, 0.5, 2.0 or 20 mg/kg orally or propranolol, 5 or 20 mg/kg orally, had no

TABLE I. Potentiation of the Antihypertensive Action of Hydralazine by Timolol. Second Series of Experiments Involving 6 × 6 Latin Square Design.

Group code	Treatment po	Maximal fall in mean arterial pressure within 24 hr after treatment, mm Hg[a]
A	Saline, 2 ml/kg + saline, 2 ml/kg	8
B	Timolol, 0.5 mg/kg + saline, 2 ml/kg	6
C	Saline, 2 ml/kg + hydralazine, 0.25 mg/kg	14
D	Saline, 2 ml/kg + hydralazine, 1.0 mg/kg	22
E	Timolol, 0.5 mg/kg + hydralazine, 0.25 mg/kg	24
F	Timolol, 0.5 mg/kg + hydralazine, 1.0 mg/kg	36

[a] Average values for 6 rats per treatment.

significant antihypertensive effect. Hydralazine lowered mean arterial pressure at 0.25 to 4 mg/kg orally. The maximal antihypertensive effect was seen at 1 hr; at higher doses the duration of action exceeded 24 hr. Timolol was tested with hydralazine at various dose combinations in a total of 76 experiments. A consistently greater effect was observed with a combination of the 2 drugs than with hydralazine alone. The maximal enhancement was seen with timolol, 0.5 mg/kg orally. The relative potency of the timolol + hydralazine combination as compared to hydralazine alone was found to be

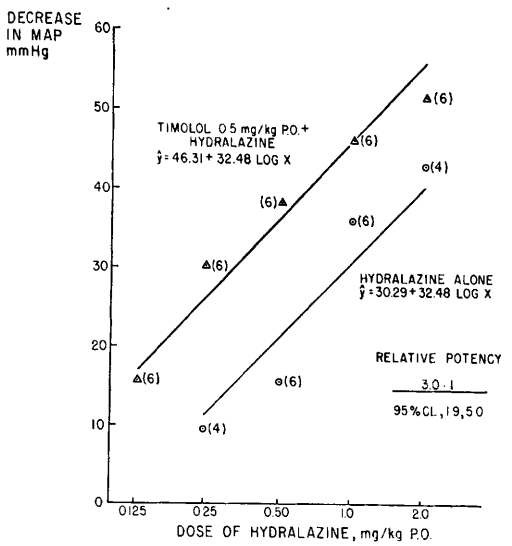

FIG. 1. Dose-response regression lines for antihypertensive effects of hydralazine alone and hydralazine + timolol, 0.5 mg/kg po in SH rats. The numbers in parentheses indicate number of animals used at each dose of hydralazine.

3.0 with 95% confidence limits of 1.9 and 5.0 (Fig. 1). The maximal antihypertensive effect of the combination was observed, as with hydralazine alone, at 1 hr after treatment. The duration of the antihypertensive action of hydralazine, 0.5 mg/kg orally, was consistently prolonged by timolol, 0.5 or 20 mg/kg orally.

Propranolol, 0.5 or 20 mg/kg orally, did not enhance the antihypertensive effect of hydralazine, 0.25, 0.5, 1 or 2 mg/kg orally.

The control heart rate of SH rats varied from 345–405 beats/min. Timolol at 0.5 mg/kg orally and higher doses and propranolol at 20 mg/kg orally consistently decreased heart rate, while hydralazine at 0.25 or 0.5 mg/kg orally usually increased heart rate. There was, however, no significant correlation between the antihypertensive and positive chronotropic effects of hydralazine. The timolol + hydralazine or propranolol + hydralazine combinations usually decreased heart rate.

Latin Square design experiments. On the basis of exploratory experiments, timolol, 0.5 mg/kg orally, significantly enhanced the antihypertensive effect of hydralazine (Fig. 1). To confirm this observation, a second series of experiments was conducted in accordance with a 6 × 6 Latin Square design. A total of 36 rats was used. They were treated with saline, timolol, hydralazine, or combinations of the two drugs. The maximal reduction of mean arterial pressure was calculated for each rat over a 24-hr period and averaged for the group (Table I). A greater antihypertensive activity of timolol + hydralazine as com-

pared to hydralazine alone was confirmed by Duncan's multiple range test for the intercomparison of treatments (Fig. 2).

Discussion. β-Adrenergic blocking drugs can be expected to antagonize increased reflex sympathetic activity initiated by hydralazine-induced peripheral vasodilation. This can theoretically lead to potentiation of the hypotensive action of hydralazine by β-adrenergic blocking agents. Under our experimental conditions, however, hydralazine did not produce cardiac acceleration consistently. Also, our observation that propranolol did not potentiate the antihypertensive action of hydralazine supports the conclusion that timolol-induced potentiation of the antihypertensive action of hydralazine is not likely to depend solely on the blockade by timolol of the reflex increase in sympathetic activity.

The possibility was considered that timolol potentiates the antihypertensive action of hydralazine by reducing the plasma renin activity which is known to be elevated by hydralazine. As recent studies from our laboratory indicate (14), timolol reduced plasma renin activity in SH rats and antagonized the hydralazine-induced increase in plasma renin activity. Propranolol, however, had similar effects on plasma renin activity so that timolol-induced potentiation of the antihypertensive action of hydralazine cannot be explained by its effect on plasma renin activity only.

The possibility that the metabolism of hydralazine is modified by timolol was not yet excluded. A highly sensitive method for detection of hydralazine in rat plasma will have to be developed, since with the available methodology (15), no hydralazine can be detected in the blood of rats following its oral administration at 0.25 or 1.0 mg/kg.

Recently some β-adrenergic blocking agents were found to lower arterial pressure in rats by repeated oral administration (16). In our experience neither timolol nor propranalol at a single oral dose had any antihypertensive activity in SH rats. In our experiments there was, therefore, no synergism between timolol and hydralazine in respect to their antihypertensive action. The observed interaction between timolol and hydralazine at single doses should be considered an example of potentiation of the pharmacological action of one drug by another.

Summary. Timolol, a new β-adrenergic blocking agent, was found to potentiate the antihypertensive effect of hydralazine in spontaneously hypertensive rats. Propranolol did not have this effect.

	B	A	C	D	E	F
F	*	*	*	*	*	
E	*	*	*	○		
D	*	*	*			
C	*	*				
A	○					
B						

FIG. 2. Statistical analysis by Duncan's multiple range test of data presented in Table I. Letters denote codes for treatment groups. Stars indicate statistical significance ($P < 0.05$) of the difference between effects of various treatments. Circles indicate absence of a significant difference. Example: the antihypertensive effect of D (saline, 2 ml/kg, + hydralazine, 1.0 mg/kg) and of F (timolol, 0.5 mg/kg, + hydralazine, 1.0 mg/kg) are significently different, whereas the effect of D and E (timolol, 0.5 mg/kg + hydralazine, 0.25 mg/kg) are not different statistically.

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