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Pharmacodynamic Interaction between Hydralazine and Phenobarbital in Normotensive and spontaneously Hypertensive Rats

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Pharmacodynamic interactions between hydralazine (HP) and phenobarbital (PB) were examined in normotensive and hypertensive rats (SHR). The hypotensive effect and the plasma concentration of HP after treatment for 7 d with the combined drugs (5 mg/kg of hydralazine hydrochloride and 2.5 mg/kg of PB) or HP alone (5 mg/kg) were measured in comparison with those in a single dose group. The relationship between the hypotensive effect and plasma level of HP was analyzed on the basis of Levy's theory. In normotensive rats, the hypotensive effect was significantly decreased and the elimination of HP from plasma was markedly enhanced after the repeated treatment with the combined drugs, as compared with the results following a single dose. The decrease in the hypotensive effect and enhancement of elimination of HP in SHR, however, were less than those in normotensive rats after repeated treatment. In a single administration of the combined drugs, PB might additively contribute to the hypotensive effect of HP. An analysis of drug interaction according to Levy's theory showed a clear-cut correlation between the plasma level and hypotensive effect of HP ($r>0.968$). The hypotensive effect declined linearly with time in each group. In normotensive rats, the extrapolated zero-time intercept (E_0) was decreased (77.0 to 53.6 mmHg) and the rate of decline of the hypotensive effect was enhanced after repeated treatment with the combined drugs as compared with that in the single dose group (-7.2 to -10.7 mmHg/h). The rate of decline after repeated treatment with the combined drugs in SHR was slightly smaller than that in the single dose group. The theoretical interpretation of the pharmacokinetic data obtained could account well for the pharmacological effects observed.

Keywords——pharmacodynamic interaction of hydralazine and phenobarbital; pharmacodynamics of hydralazine and phenobarbital; relationship between hypotensive effect and plasma level of hydralazine; hydralazine; antihypertensive drug

Hydralazine (1-hydrazinophthalazine, HP), a potent peripherally acting vasodilator, has been used for the treatment of essential hypertension.[1-3] Generally, hypotensors, including HP, which are applied to clinical therapy are apt to be repeatedly administered and to be frequently used with sedatives and other drugs. The era when phenobarbital (PB) was virtually the only drug recommended for daytime sedation has passed, but nevertheless, the barbiturate is still employed as a sedative. The coadministration of HP and PB may enhance the clinical effect as compared with administration of HP alone, because PB indirectly helps to reduce the blood pressure due to the sedation of the medullary vasomotor center.[4] On this basis, the combined prescription involving both drugs was widely used for patients with hypertension for many years.

However, since barbiturates, especially PB, are known to be potent inducers of liver drug-metabolizing enzymes,[5,6] it has been suggested that chronic treatment with both HP and PB may change the hypotensive effect and disposition of HP owing to the increase in drug-metabolizing enzymes. Thus, the coadministration of drugs and PB has gradually become less common.

There are several reports on metabolism[7,8] and pharmacokinetics[9-11] of HP in man and experimental animals, but little is known about the effect of repeated treatment with the combined drugs, HP and PB, on the hypotensive effect, or about the therapeutic validity of the coadministration of HP and PB. On the other hand, some disagreement exists regarding

the relationship between the hypotensive effect of HP and its plasma level.[12-15]

The aim of this study was to clarify the contribution of PB to the hypotensive effect of HP and the relationship between the plasma level and hypotensive effect of HP based on pharmacodynamic studies. The data reported here include our observations on the hypotensive effect and plasma level of HP after single and repeated (7 d) administrations of the combined drugs to normotensive rats and spontaneously hypertensive rats (SHR).

Experimental

Materials——HP and PB were obtained from Ciba-Geigy Co., Ltd. and Hoei Yakko Co., Ltd., respectively. 1-Hydrazino-4-methyl-phthalazine, an internal standard for gas liquid chromatography (GLC), was a gift from Ciba-Geigy Co., Ltd. All chemicals used were of special grade.

Animals and Treatment——Male normotensive Wistar rats weighing 90—100 g and male Okamoto spontaneously hypertensive rats (SHR) weighing 250—350 g were used throughout. The animals were divided at random into 3 groups. A) Controls were treated for 7 d with daily intraperitoneal (*i.p.*) injection of saline (Control). B) Animals were treated for 6 d with *i.p.* injection of saline and with a single *i.p.* injection of the combined drugs (5 mg/kg of HP and 2.5 mg/kg of PB) or HP (5 mg/kg) alone on day 7 (Single). C) Animals were treated for 7 d with daily *i.p.* injection of the combined drugs at the same dose as in the single group (Repeated). The drugs were dissolved in sterilized 15% propylene glycol–saline mixture. Since the single and repeated administrations of 15% propylene glycol–saline mixture had no effect on the hypotensive effect or the plasma concentration of HP in rats, the animals of the control and single groups received saline instead of 15% propylene glycol–saline mixture.

Measurement of Blood Pressure——Blood pressure was measured by the tail pulse pick-up method using a Natume KN-0091 apparatus after warming the animals in a heated box maintained at 65 ± 1°C for 2 min. The measurement was carried out 0.75, 1.5, 3 and 4.5 h after the final injection of the drug in a room at 25°C.

Determination of HP in Plasma——Animals were fixed on the operating table without anesthesia. Blood specimens were collected into the heparinized syringes from the femoral vein and immediately plasma was obtained by centrifugation at 2500 rpm for 10 min. HP in plasma was determined according to the method of Zak *et al.*,[16] which is a selective analysis method for HP (unchanged HP) or its acid-labile conjugates, by GLC using a Hitachi 163 gas chromatograph with an electron capture detector and a 3 mm × 1 m glass column packed with 3% OV-225 on Chromosorb W-HP (80—100 mesh).

Pharmacokinetic Analysis——The logarithmic concentration of HP in plasma was plotted against time after administration. From the plotted curve, the elimination rate constant was calculated by means of the least-squares method.

Pharmacological and Statistical Analysis——The relationship between the observed hypotensive effect and plasma concentration of HP was analyzed according to a theory, reviewed by Levy,[17] for relating kinetic data to pharmacological effects, by means of the relationships shown in the equations:

$$E = m \log C + e \quad (1)$$

$$E = E_0 - (m \cdot K_{el}/2.303)\, t \quad (2)$$

where E is the intensity (absolute reduction of blood pressure in our case) of the hypotensive effect, C is the HP concentration in plasma, m is the slope of a plot of response *vs.* logarithmic plasma concentration, e is the intercept of the line on the E axis, E_0 is the extrapolated zero-time intercept, K_{el} is the elimination rate constant of HP, t is the time (h) after administration and $-(m \cdot K_{el}/2.303)$ is the rate of decline of the hypotensive effect. The data were compared by an analysis of variance. When the analysis indicated that a significant difference existed, the means of the treated groups were compared with the control mean by Student's t-test with $p<0.05$ as the criterion of significance. All averaged data are presented as the mean ±S.D. Correlation analyses were carried out by the least-squares linear regression method. Correlation coefficients were examined for significance ($p<0.05$) by means of the t-test.

Results

Hypotensive Effect following Single or Repeated Administration of the Combined Drugs in Normotensive and Hypertensive Rats

Animals received a single or 7-d's treatment with the combined drugs, and the blood pressure was measured following the final administration. The results are shown in Fig. 1 for normotensive rats (a) and SHR (b). In normotensive rats, a significant difference in hypotensive effect between the single and repeated dosing groups was observed; the hypotensive

effect was more markedly decreased in the repeated dosing group during the experimental period than after a single dosing ($p<0.005$). At 4.5 h after administration, the hypotensive effect in the single dosing group was still apparent, whereas in the repeated dosing group the effect had almost disappeared.

In SHR, a relatively slight but significant difference in hypotensive effect was observed between the single and repeated treatment groups. Although the hypotensive effect in the repeated dosing group was less than that in the single dosing group, the effect continued for a relatively long time in both groups, and the decrease in the effect following repeated treatment of the combined drugs was not as marked as in the case of normotensive rats.

In the animals used in these experiments, there were no statistically significant differences in blood pressure at 0 time among the groups.

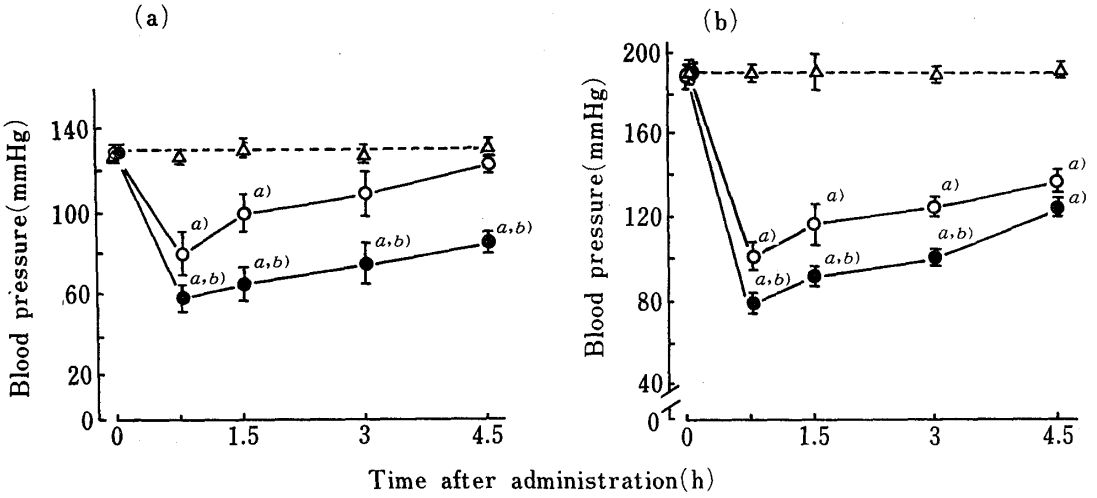

Fig. 1. Effect of Combined Drugs after Single and Repeated Administrations on Blood Pressure in Normotensive Rats (a) and Spontaneously Hypertensive Rats (b)

△; control, ●; single, ○; repeated. Blood pressure was measured after administration of the drugs (7.5 mg/kg body weight, *i.p.*). Each point represents the mean ± S.D. of 6 rats (normotensive rats) or 4—5 rats (SHR). *a*) $p<0.005$ in control *vs.* single or repeated, *b*) $p<0.005$ in single *vs.* repeated.

Hypotensive Effect following Single or Repeated Administration of Hydralazine in Normotensive Rats

Each group received single or repeated administration of HP alone (5 mg/kg) and blood pressure was measured following the final administration. The results are shown in Fig. 2. No significant difference in hypotensive effect between the single and repeated dosing groups beyond 1.5 h after administration was observed, though a slight but significant difference was observed at 0.75 h after dosing between the two groups ($p<0.005$). This indicates that repetition of HP treatment did not in itself significantly decrease the hypotensive effect.

When the hypotensive effect of HP alone was compared with that of the combined drugs in single dosing, the effects at 0.75 and 3 h after a single injection of HP alone were about 58

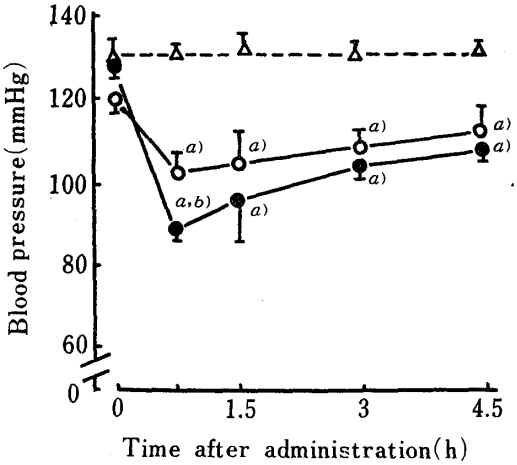

Fig. 2. Effect of Single and Repeated Administrations of HP alone on Blood Pressure in Normotensive Rats

△; control, ●; single, ○; repeated. Each point represents the mean ± S.D. of 4 rats. *a*) $p<0.005$ in control *vs.* single or repeated, *b*) $p<0.005$ in single *vs.* repeated.

and 44%, respectively, of those obtained by a single administration of the combined drugs. This suggests that in a single treatment, PB as a component of the combined drugs contributes substantially to the hypotensive effect of HP.

Plasma Concentration of Hydralazine after Single or Repeated Administration of the Combined Drugs in Normotensive and Hypertensive Rats

The animals were treated by the procedure described in the experimental section and the logarithmic concentrations of HP in the plasma after single or repeated administration of the combined drugs were measured for normotensive rats and SHR (Fig. 3a and b, respectively) as a function of the time after administration. The plasma decay curve of HP could be described by a single first-order function. In normotensive rats, the plasma levels of HP at each time in the repeated dose group were significantly lower than those in the single treatment group; in particular, the levels at 3 and 4.5 h after dosing in the repeated treatment group were below 50% of those in the single dose group. The elimination rate constant for HP in the repeated dosing group was significantly higher than that in the single dosing group (single, $0.205\pm0.085\ h^{-1}$; repeated, $0.446\pm0.078\ h^{-1}$; $p<0.005$).

In SHR, the plasma levels of HP after repeated treatment were slightly lower than those in the single dose group at each time, and the elimination rate constant was slightly higher than that in the single dose group (single, $0.273\pm0.059\ h^{-1}$; repeated, $0.371\pm0.058\ h^{-1}$; no significance). These results are in accord with the difference of the hypotensive effects after single and repeated treatment in both normotensive rats and SHR. It should be noted that the plasma levels of HP in SHR after a single administration of the combined drugs are lower anth hotse in normotensive rats, but there is no significant difference in levels between both types of rats after repeated treatment.

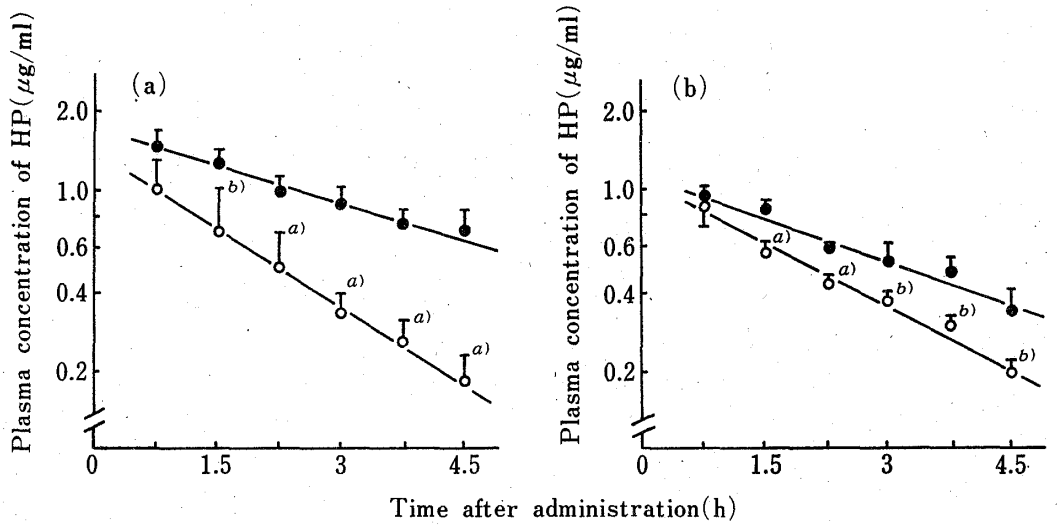

Fig. 3. Concentration of Hydralazine in Plasma after Single and Repeated Administrations of Combined Drugs in Normotensive Rats (a) and Spontaneously Hypertensive Rats (b)

●; single, ○; repeated. Hydralazine was determined after administration of the drugs (7.5 mg/kg, *i.p.*). Each point represents the mean ± S.D. of 5 rats (normotensive rats) or 4 rats (SHR). *a*) $p<0.005$ in single *vs.* repeated, *b*) $p<0.025$ in single *vs.* repeated.

Relationship between Hypotensive Effect and Plasma Level of HP

The relationship between the hypotensive effect observed after single or repeated administration of the combined drugs and the plasma levels of HP (unchanged HP) is shown in Fig. 4. In both normotensive rats and SHR, the relationship between the effectiveness (E) (reduction of blood pressure) and the logarithmic concentration of HP in plasma ($\log C$) was linear and could be described by the equation, $E=m \log C+e$, and there was a significant correlation

between log C and the effect in each group. However, the slope m and intercept e tend to decrease on repeated treatment in both normotensive rats and SHR (Table I). These results indicate that repeated treatment produced the depression of the hypotensive effect and the effect of HP is significantly related to the concentration of the parent drug in plasma in rats.

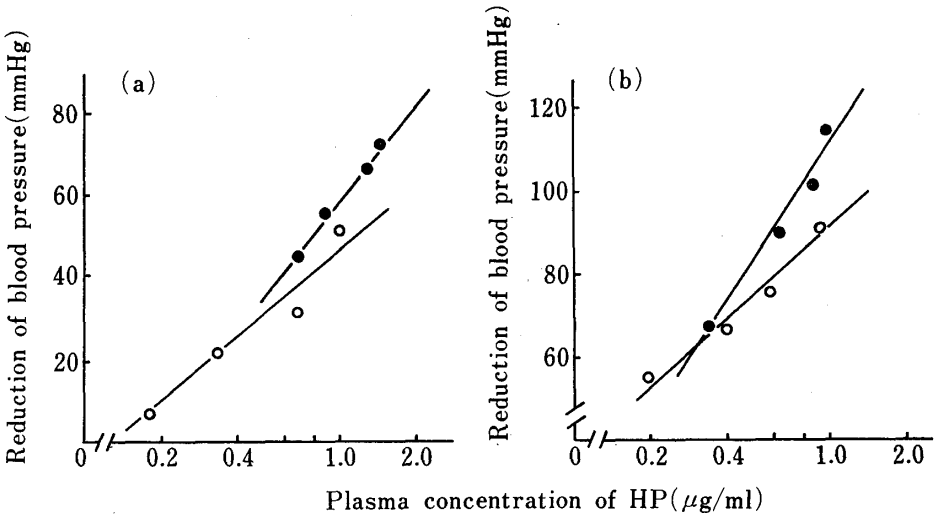

Fig. 4. Relationships between Plasma Concentration of Hydralazine and Hypotensive Effect in Normotensive Rats (a) and Spontaneously Hypertensive Rats (b)

●; single, ○; repeated. Each point represents experimental data taken from Fig. 1 and Fig. 3. A plot of effect (E) *vs.* log C can be represented by the equation $E=m \log C+e$.

TABLE I. Relationships between Hypotensive Effect and log Plasma Hydralazine Concentration

Animals	Single	Repeated
Normotensive rats	$E=81.2 \log C+58.9$ ($r=0.997$)	$E=53.7 \log C+46.2$ ($r=0.968$)
SHR	$E=92.3 \log C+111.6$ ($r=0.975$)	$E=53.8 \log C+91.9$ ($r=0.986$)

Equations were obtained from observed effect-log HP levels by the least-squares method (see Fig. 4).

When the hypotensive effect obtained from the data in Fig. 1 was represented as a function of time, the drug effect declined linearly with or at a zero-order rate in both normotensive rats and SHR, as shown in Fig. 5. In normotensive rats, the hypotensive effect (E) could be described well by the equations $E=77.0-7.2\,t$ for the single dose group, and $E=53.6-10.7\,t$ for the repeated dose group. After repeated treatment with the combined drugs, the value of E_0 decreased and the mean rate of decline of the effect was in general 1.5 times faster than that obtained after a single dosing. In SHR, the time-course of hypotensive effects could be represented by the equations $E=122.9-11.8\,t$ and $E=94.9-8.9\,t$ for the single and repeated dose groups, respectively. The value of E_0 in the repeated dose group was significantly decreased as compared with that in the single dosing group, whereas, the mean rate of decline of the drug effect was rather less than that in the single dose group.

When the rate of decline of drug effect, $-(m \cdot K_{el}/2.303)$, whichwas obtained from the slope m of a plot of the effect *vs.* log plasma HP level and from the elimination rate constant K_{el}, was compared with the rate obtained from actually observed effect-time data depicted in Fig. 5, very good agreement was found between the calculated and observed values in each group as

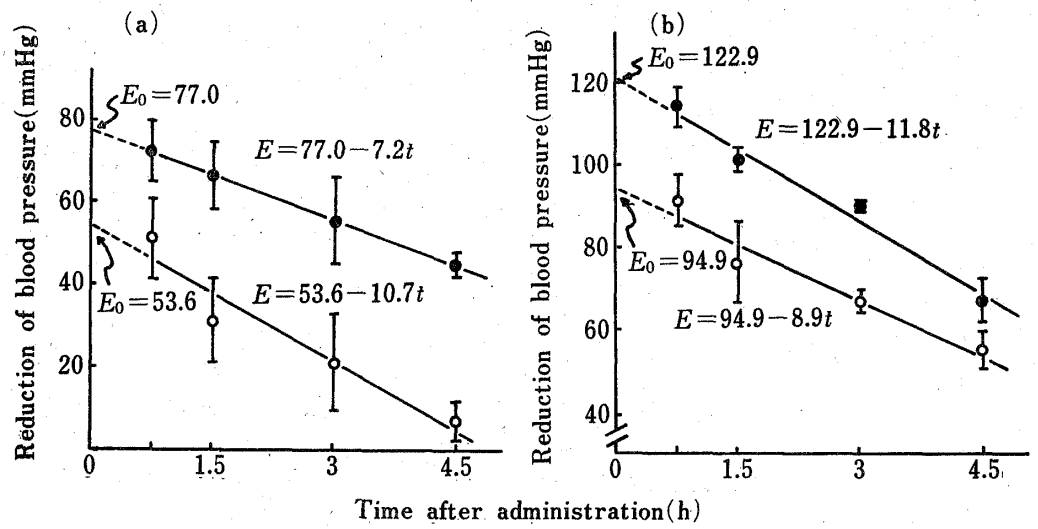

Fig. 5. Relationships between Hypotensive Effect and Time in Normotensive Rats (a) and Spontaneously Hypertensive Rats (b)

●; single, ○; repeated. The data (mean ± S.D.) were obtained from 6 rats (normotensive rats) or 4—5 rats (SHR).

Table II. Rate of Decline of Hypotensive Effect

Rats	Treatment	From observed effect-time curve[a)]	From Levy's theory[b)]
Normotensive rats	Single	− 7.2	− 7.2
	Repeated	−10.7	−10.4
SHR	Single	−11.8	−10.9
	Repeated	− 8.9	− 8.7

a) Calculated from actually observed effect-time curve for 6 rats (normotensive rats) or 4—5 rats (SHR).
b) Calculated from the slope (m) of effect-log concentration curve (see Fig. 4) and the elimination rate constant (K_{el}) calculated from the plasma concentration–time curve (see Fig. 3).

shown in Table II. Therefore, it is strongly suggested that the effects of HP are correlated with the plasma HP levels and consequently with the elimination rate constant of HP. Additional evidence is provided by the fact that there was very good agreement (r=0.989 for normotensive rats and r=0.986 for SHR) between the observed hypotensive effect and the effect calculated by means of Levy's equation (2). The elimination rate constants obtained from the rate of decline of the pharmacological effect, calculated from the data shown in Fig. 5, were 0.204 and 0.459 h^{-1} for the single and repeated dosing groups, respectively, in normotensive rats, and 0.294 and 0.381 h^{-1} for both groups, respectively, in SHR. These values agree very well with the constants (K_{el}) obtained from the plasma levels.

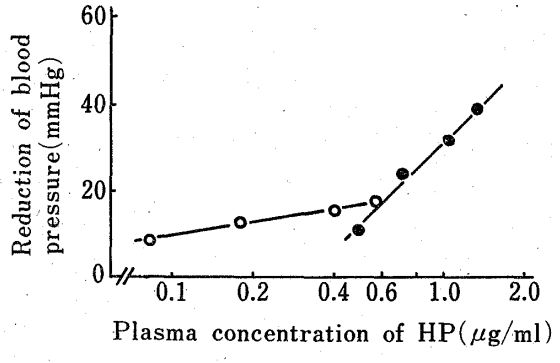

Fig. 6. Relationships between Plasma Concentration of Hydralazine and Hypotensive Effect after Administration of Hydralazine alone in Normotensive Rats

●; single, ○; repeated. Animals received HP (5 mg/kg) alone. Each point represents the mean of 4—5 rats.

For single and repeated administrations of HP alone in normotensive rats, the relationship between the effectiveness obtained from Fig. 2 and the logarithmic concentra-

tion of HP could be described by the following equations; $E=61.9 \log C+31.3$ and $E=10.3 \log C+20.3$, respectively. These results also demonstrate that the effects of HP are highly correlated with the plasma levels.

Discussion

The present study was carried out to clarify whether or not PB enhances the hypotensive effect of HP in rats additively even after repeated treatment and to elucidate the relationship between the effect and the plasma concentration of HP following coadministration of this hypotensive agent and PB. The reason why rats of different ages were used in this experiment was to determine sensitively the pharmacodynamic response to the combined drugs in young (normotensive) animals and to measure clearly the hypotensive effect of the drugs on adult rats with hypertension.

The present study demonstrated that repeated treatment with the combined drugs resulted in a significantly decreased hypotensive effect in normotensive rats, but the effect was decreased only comparatively slightly in SHR (Fig. 1). Consequently, repeated treatment in normotensive rats with the combined drugs results in the development of apparent tolerance to the drug. In normotensive rats, treatment for 7 d with the combined drugs markedly increased the elimination of HP from plasma, while the same treatment in SHR did not increase it so much (Fig. 3). Thus, the significant decrease of hypotensive effect after the repeated treatment with the combined drugs can be mainly ascribed to enhanced plasma clearance of HP. It is presumed that the differences in hypotensive effect and elimination behavior between normotensive rats and SHR may be due to differences in the response of the blood flow of the liver and kidney to the drugs, especially PB, and in the inducibility of drug-metabolizing enzymes.

It is of interest that the hypotensive effect of the combined drugs in SHR is more potent than that in normotensive rats in spite of the lower plasma levels of HP (Fig. 1 and 3), since this indicates that SHR may be more sensitive to the drugs. Thus, it remains a possibility that HP administration may exert a significant hypotensive effect on patients with abnormally elevated blood pressure. On the other hand, the hypotensive effect after single and repeated treatment with HP alone was maintained for a longer time in normotensive rats.

The plasma disappearance of HP in rats seems to follow first-order kinetics (Fig. 3). This result is in agreement with the data obtained by many workers.[11,18,19]

In an attempt to obtain an insight into the elimination and response kinetics of HP, we applied a theory presented by Levy.[17] A high correlation was found between the plasma concentration and hypotensive effect of HP in each group even after repeated coadministration of HP and PB (Fig. 4). The observation that there were no significant differences between the elimination rate constant, K_{el}, actually observed and the rate constant calculated from Levy's equation also indicates that the hypotensive effect of HP is related to the plasma level of unchanged HP. Thus, these results confirm the applicability of Levy's theory[17] to the pharmacodynamics of HP.

Zacet and Koch-Weser[13] showed that the mean reduction of arterial pressure in 20 patients was proportional to their HP plasma levels at the time of blood pressure determination. On the contrary, Shepherd *et al.*[14] and Talseth[12] reported that there was no correlation between the duration of the hypotensive effect of HP and its plasma level in man, and O'Malley *et al.*[15] reported that the duration of the hypotensive action of HP exceeded that predicted from the rate of elimination of the parent compound from the plasma, and that the half-life of the return of blood pressure to pre-treatment levels after cessation of a 2-week treatment in hypertensive patients amounted to 30—140 h (mean 97.5 h). The cause of the disagreement is considered to be in the determination methods, which are not sufficiently sensitive and specific to monitor plasma levels of unchanged HP. Our results strongly suggest that the hypotensive

effect of HP is very well correlated with the plasma level of unchanged HP after administration of HP alone or in combination with PB. This is an important finding for the treatment of hypertension and implies the need to measure routinely plasma levels of HP in treated hypertensive patients.

The elimination of PB from plasma was shown to be extremely slow; the elimination rate constant was 0.0024 min^{-1} in rats.[20] This suggests that the elimination of PB from the blood after administration of this drug may be extremely slow in both single and repeated dose groups. Consequently, PB may affect the elimination of HP from plasma, probably due to the inductive effect or increased clearance.

When the animals were treated with a single administration of both HP and PB, the hypotensive effect was more enhanced than after HP alone; suggesting a very effective cooperative action of the two drugs. However, repeated treatment with both drugs results in a significantly decreased hypotensive effect in normotensive rats, but a smaller decrease in the effect was seen in SHR. Therefore, the combined drugs may still be effective for the therapy of hypertension.

In conclusion, treatment for 7 d with the combined drugs significantly decreased the hypotensive effect, mainly due to enhanced elimination of HP from the plasma, although the decrease in the effect in SHR was not as great as that in normotensive rats. A strongly hypotensive effect was found in SHR despite lower plasma HP levels as compared with those in normotensive rats. The hypotensive effect of HP was significantly correlated with the plasma concentration of unchanged HP and the relationship between the plasma level and hypotensive effect of HP could be very well described by Levy's theory.[17] PB in the combined drugs contributed additively to the hypotensive effect of HP after a single dosing. However, the contribution after repeated treatment was decreased. Nevertheless, the use of the combined drugs for the therapy of hypertension may still be valid, on the basis of the above considerations.

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References and Notes

1) P. Blaquier and S.W. Hoobler, *Am. J. Med. Sci.*, **238**, 740 (1959).
2) J.D. Morrow, H.A. Schroeder and H.M. Perry, *Circulation*, **8**, 829 (1953).
3) J. Koch-Weser, *New Engl. J. Med.*, **295**, 320 (1976).
4) S.C. Harvey, "The Pharmacological Basis of Therapeutics," New York, 1975, p. 102.
5) A.H. Conney, C. Davision, R. Gastel and J.J. Burns, *J. Pharmacol. Exp. Ther.*, **130**, 1 (1960).
6) S.A. Cucinell, A.H. Conney, M. Sanser and J.J. Burns, *Clin. Pharmacol. Ther.*, **6**, 420 (1965).
7) Z.H. Israili and P.G. Dayton, *Drug Metab. Dispos.*, **6**, 283 (1977).
8) W.M. McIssac and M. Kanda, *J. Pharmacol. Exp. Ther.*, **143**, 7 (1964).
9) T. Talseth, *Europ. J. Clin. Pharmacol.*, **10**, 183 (1976).
10) T. Talseth, *Europ. J. Clin. Pharmacol.*, **10**, 395 (1976).
11) M.M. Reidenberg, D. Drayer, A.L. DeMarco and C.T. Bello, *Clin. Pharmacol. Ther.*, **14**, 970 (1973).
12) T. Talseth, *Clin. Pharmacokin.*, **2**, 317 (1977).
13) R. Zacet and J. Koch-Weser, *Clin. Pharmacol. Ther.*, **13**, 420 (1972).
14) A.M.M. Shepherd, *Res. Commun. Chem. Pathol. Pharmacol.*, **26**, 129 (1979).
15) K. O'Malley, J.L. Segal, Z.H. Israili, M. Boles, J.L. McNay and P.G. Dayton, *Clin. Pharmacol. Ther.*, **18**, 581 (1976).
16) S.B. Zak, G. Lukas and G. Gilleran, *Drug Metab. Dispos.*, **5**, 116 (1977).
17) G. Levy, *Clin. Pharmacol. Ther.*, **7**, 362 (1966).
18) D.W. Schneck, J.S. Sprouse, K. Miller, J.E. Vary, F.O. Dewitt and A.H. Hayer, *Clin. Pharmacol. Ther.*, **24**, 714 (1978).
19) D.D. Shen, J.P. Hosler, R.L. Schroder and D.L. Azarnoff, *J. Pharmacokinet. Biopharm.*, **8**, 53 (1980).
20) Y. Lin, S. Awazu, M. Hanano and H. Nogami, *Chem. Pharm. Bull.*, **21**, 2749 (1973).