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Report

Effect of Hydralazine on the Elimination of Antipyrine in the Rat

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The concomitant administration of hydralazine with metoprolol or propranolol substantially increases the oral bioavailability of these beta-blockers, presumably via reduction of the first-pass effect. It has been suggested that this effect may be secondary to a decrease in the intrinsic clearance of propranolol, possibly by inhibition of oxidative metabolism. To examine the possibility that hydralazine alters oxidative metabolism *in vivo*, the effect of hydralazine on the pharmacokinetics of antipyrine was examined in the rat. The oral administration of hydralazine hydrochloride, 7.5 mg/kg, 15 min prior to antipyrine administration reduced antipyrine clearance from 9.66 ± 1.18 to 8.19 ± 0.76 ml/min/kg ($P < 0.05$). Hydralazine was observed to cause substantial hypothermia. The study was repeated in temperature-regulated animals and no alteration in antipyrine clearance was found. Two doses of hydralazine in temperature-regulated rats also failed to alter antipyrine clearance. Thus, it appears that the effect of hydralazine on antipyrine clearance is secondary to the hypothermic effect of hydralazine and not due to a direct inhibition of cytochrome P-450-mediated enzyme activity.

KEY WORDS: antipyrine; drug metabolism; hydralazine; hypothermia; pharmacokinetics.

INTRODUCTION

The coadministration of metoprolol or propranolol with hydralazine in humans results in substantial increases (30–70%) in the oral AUC of these beta-blockers (1–5). The mechanism(s) of this interaction remains unclear. It was postulated that this effect might be secondary to a transient increase in hepatic blood flow resulting in a reduction in presystemic elimination with no significant influence on systemic clearance (5,6). However, it has been shown that hydralazine does not significantly alter hepatic blood flow in humans at doses found to increase metoprolol and propranolol bioavailability (7,8). This suggests that a transient change in hepatic blood flow is not the primary mechanism for this interaction.

Another possible explanation for this phenomenon could be that hydralazine directly reduces the apparent intrinsic hepatic clearance of these beta-blockers. This is supported by a recent investigation showing that hydralazine alters the hepatic extraction of propranolol in the dog during constant hepatic blood flow (9).

While there are several mechanisms by which hydralazine could reduce the intrinsic clearance of propranolol, inhibition of oxidative metabolism deserves prime consideration. Indeed, there is evidence that hydralazine inhibits microsomal drug metabolism *in vitro* (10,11). To examine the potential that hydralazine inhibits oxidative metabolism *in vivo*, we examined the effect of hydralazine administration on the pharmacokinetics of antipyrine in the rat. Antipyrine is eliminated primarily by cytochrome P-450-dependent metabolism, exhibits a low intrinsic hepatic clearance, and is not significantly bound to plasma proteins. Changes in its total body clearance, therefore, can be concluded to result from changes in P-450-dependent drug metabolism.

MATERIALS AND METHODS

Chemicals. Antipyrine was purchased from Aldrich Chemical Company (Milwaukee, Wis.). Hydralazine hydrochloride and phenacetin (internal standard) were purchased from Sigma (St. Louis, Mo.). Propylene glycol and ethyl ether were obtained through VWR Scientific (Chicago, Ill.). All chemicals were used as received.

Animals and Treatment. Male Sprague–Dawley rats (weighing 166 to 239 g) had an indwelling cannula implanted in the right jugular vein under light ether anesthesia 1 day prior to antipyrine administration (12). Animals were individually housed in plastic metabolism cages. Antipyrine (20 mg/kg) was dissolved in physiologic saline (to a final concentration of 10 mg/ml) and infused through the cannula at a rate of 0.34 ml/min. Serial blood samples (0.25 ml) were obtained through the cannula over a 5-hr period. Blood was collected in plastic syringes and transferred to heparinized glass capillary tubes. Plasma was separated by centrifugation and stored at −20°C until analyzed by an HPLC method described previously (13). Preliminary experiments in which animals received hydralazine without antipyrine revealed that hydralazine administration did not result in any interfering peaks.

Animals received hydralazine hydrochloride, 7.5

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mg/kg, or vehicle orally as indicated in the results. Hydralazine hydrochloride was dissolved in a solution of 30% propylene glycol in 0.9% saline to a final concentration of 3 mg/ml immediately prior to administration. Preliminary experiments indicated that this diluent had no significant effect on the pharmacokinetics of antipyrine (data not shown).

Body temperature was monitored in all animals rectally using a YSI Tele-thermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio). In some animals body temperature was maintained by a heat lamp. The proximity of the lamp to the animal was adjusted throughout the experiment to maintain body temperature at the baseline reading.

Data Analysis. The initial antipyrine plasma concentration was calculated from the zero intercept of the plasma concentration–time curve using unweighted nonlinear least-squares regression. Statistical moment analysis was used to obtain the model-independent pharmacokinetic parameters (14). Pharmacokinetic parameters and body temperatures between control and treatment groups were compared using the two-tailed Student's t test. A value of $P < 0.05$ was considered statistically significant. Data are presented as mean ± standard deviation.

RESULTS

The mean antipyrine plasma concentration versus time profile in control animals and those pretreated with a single oral dose of hydralazine hydrochloride, 7.5 mg/kg, 15 min prior to antipyrine administration is illustrated in Fig. 1. The data in Table I indicate that hydralazine pretreatment resulted in a 15% reduction in antipyrine clearance (from 9.66 ± 1.18 to 8.19 ± 0.76 ml/min/kg; $P < 0.05$) and a 14% increase in half-life (from 67.2 ± 6.8 to 76.9 ± 4.9 min; $P < 0.02$). Animals pretreated with hydralazine also exhibited a significant reduction in body temperature throughout the period of blood sampling (Fig. 2).

Table I. Effect of Oral Hydralazine Hydrochloride, 7.5 mg/kg, on the Pharmacokinetics of Antipyrine in the Rat

		Pharmacokinetic parameter[b]		
Treatment[a]	N	CL (ml/min/kg)	$t_{1/2}$ (min)	V_{dss} (ml/kg)
Control	6	9.66 (1.18)	67.2 (6.8)	932 (71)
Hydralazine	6	8.19 (0.76)*	76.9 (4.9)**	899 (38)

[a] Hydralazine hydrochloride or vehicle was given 15 min prior to antipyrine administration.
[b] Data are presented as mean (± SD).
* $P < 0.05$.
** $P < 0.02$.

Because hypothermia could account in part for the decreased antipyrine clearance, another study was performed in which body temperature was maintained by a heat lamp. The data in Table II demonstrate that hydralazine had no significant effect on antipyrine pharmacokinetics in thermally controlled animals. Body temperature was not significantly different between the two groups at any time during the experiment (Fig. 3). Furthermore, when hydralazine was administered at the same dose both 15 min prior to and 120 min after antipyrine administration to thermally controlled rats, there was no alteration in antipyrine pharmacokinetics (Table II).

DISCUSSION

The potential for an interaction between vasodilators and the beta-blockers metoprolol and propranolol was first suggested in a theoretical discussion proposing a potential

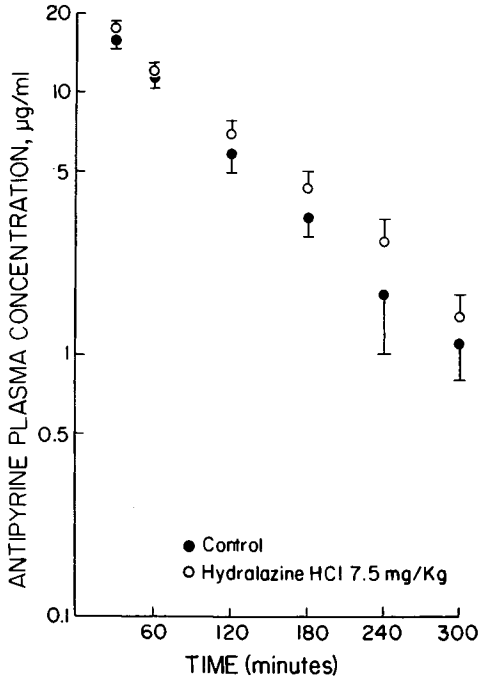

Fig. 1. Mean antipyrine plasma concentration versus time profile in animals receiving vehicle (●) or hydralazine hydrochloride (○), 7.5 mg/kg, 15 min prior to antipyrine administration. Bars represent 1 SD.

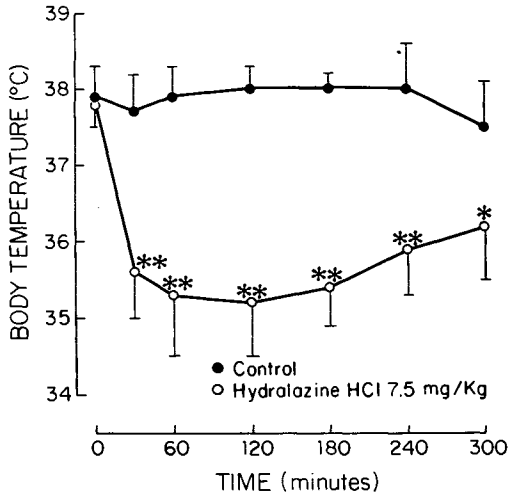

Fig. 2. Mean body temperature in animals receiving vehicle (●) or hydralazine hydrochloride, 7.5 mg/kg (○), 15 min prior to antipyrine administration. Time indicated is time after hydralazine administration. Bars represent 1 SD. (*) $P < 0.01$; (**) $P < 0.001$

Table II. Effect of Oral Hydralazine, 7.5 mg/kg, on the Pharmacokinetics of Antipyrine in Thermally Controlled Rats

Treatment[a]	N	Pharmacokinetic parameters[b] CL (ml/min/kg)	$t_{1/2}$ (min)	V_{dss} (ml/kg)
Control	8	8.79 (1.01)	62.7 (9.3)	795 (68)
Hydralazine ×1	8	8.11 (1.46)	71.5 (16.3)	822 (67)
Control	7	8.11 (1.66)	60.6 (8.0)	703 (126)
Hydralazine ×2	7	8.10 (1.96)	69.3 (12.7)	779 (86)

[a] Hydralazine or vehicle was given 15 min prior to antipyrine in both studies and again at 2 hr in the multiple-dose (designated ×2) study.

[b] Data are presented as mean (± SD).

mechanism for the increased bioavailability of these beta-blockers when administered with food (6). Computer simulations indicated that a transient increase in hepatic blood flow should result in an increase in the oral area under the curve (AUC) of high-clearance drugs similar to that seen when these compounds were administered with food (15). Since vasodilators may produce similar changes in hepatic blood flow, it was suggested that they might also alter the bioavailability of high-clearance drugs (6). Subsequently, hydralazine was found to increase the oral AUC of propranolol, as predicted (5).

Recent experimental evidence, however, has questioned the blood-flow hypothesis as a mechanism for these interactions (16–18). In particular, it has been shown that hydralazine does not significantly alter hepatic blood flow in humans at doses that increase metoprolol and propranolol bioavailability (7,8). In addition, it has recently been demonstrated that hydralazine decreases the hepatic extraction of propranolol in the dog even when the hepatic blood flow remains unchanged (9). These data suggest that these interactions are caused by some mechanism other than simple changes in hepatic blood flow. One possibility would be a reduction in the apparent intrinsic clearance. Such a reduction could result from either reduced hepatic uptake or reduced metabolism. The observation that hydralazine inhibits drug metabolism *in vitro* suggests that this agent could be an inhibitor of oxidative metabolism *in vivo* (10,11). In an effort to examine the magnitude of this phenomenon *in vivo*, we determined the effect of hydralazine on the disposition of antipyrine in the rat.

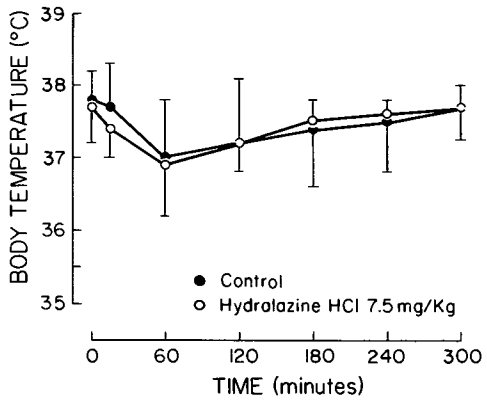

Fig. 3. Mean body temperature in animals receiving a single dose of vehicle (●) or hydralazine hydrochloride, 7.5 mg/kg (○), 15 min prior to antipyrine administration and placed under a heat lamp to maintain body temperature. Time indicated is time after hydralazine administration. Bars represent 1 SD.

Single-dose administration of hydralazine was found to result in a significant reduction in the clearance of antipyrine. As a compound with a low intrinsic clearance, the systemic clearance of antipyrine is independent of hepatic blood flow (19). This observation, therefore, suggested that hydralazine may have inhibited the metabolism of antipyrine. We also found, however, that this dose of hydralazine resulted in a substantial reduction in body temperature. Hydralazine-induced hypothermia in rodents has been reported previously (20). Since hypothermia itself has been shown to alter hepatic drug metabolism (21), it is possible that the effect of hydralazine on antipyrine clearance was due, in part, to hypothermia and not a result of a direct interaction of hydralazine on P-450 cytochromes. Indeed, when the study was repeated in animals whose body temperature was maintained, hydralazine failed to cause a significant reduction in antipyrine clearance.

Thus, the data in this report indicate that while hydralazine does decrease antipyrine clearance in the rat, this effect is due to the hypothermic effects of hydralazine. Since hypothermia generally does not occur in humans after hydralazine administration, it seems unlikely that this is the mechanism for the alteration in the presystemic elimination of metoprolol and propranolol when coadministered with hydralazine.

These observations do not, however, rule out the possibility that hydralazine may have its effect on metoprolol and propranolol through a reduction in the intrinsic hepatic clearance of these compounds. First, the effect of hydralazine on intrinsic clearance may be a very transient phenomenon. A transient effect would be sufficient to alter the presystemic elimination of a drug such as propranolol but may not be adequate to affect the time-averaged systemic clearance of a drug such as antipyrine. In an effort to examine for a possible transient effect of hydralazine on drug metabolism, we examined the effect of multiple-dose administration (see Table II). Second, in view of the multiplicity of P-450 cytochromes, hydralazine may alter only those isozymes responsible for propranolol metabolism and not those which are responsible for antipyrine metabolism. Recently, food has been shown to alter the conjugation of propranolol (22). Hydralazine could alter the first pass of propranolol via alterations in conjugation. This would not, however, explain its effect on metoprolol, a drug which does not undergo significant conjugation (23). Alternatively, hydralazine may exhibit its effect on these beta-blockers by a reduction in their hepatic uptake, without altering enzymatically controlled metabolic reactions. It would appear that an isolated perfused organ system where extraction can be determined directly would be the best way to elucidate these various potential mechanisms.

Of importance to the interpretation of this study is the relevance of the dose of hydralazine administered in the rat to doses that increase the bioavailability of propranolol in

humans. The dose of hydralazine administered in the present study has been previously shown to substantially reduce blood pressure in spontaneously hypertensive rats (24). More importantly, this dose produced an average peak plasma hydralazine concentration of 1000 ng/ml in the rat (24), while an oral dose of 1 mg/kg produces peak plasma hydralazine concentrations in humans which range from 16 to 208 ng/ml (25). Thus, the dose used in the present study produced somewhat higher plasma concentrations in the rat than those normally observed in humans.

In conclusion, it appears that the effect of hydralazine on oxidative drug metabolism in the rat is secondary to the hypothermic effect of the drug. This indicates that hydralazine is probably not an enzyme inhibitor per se. Since hypothermia does not usually occur in humans after hydralazine administration, it is unlikely that hydralazine will affect the hepatic metabolism of low-intrinsic clearance drugs.

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