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Clonidine effects on disposition of xenobiotics in rats: inhibited elimination of flow-limited but not extraction-limited agents

Zvi Ben-Zvi & [1]*Aryeh Hurwitz

Ben-Gurion University of the Negev, Faculty of Health Sciences, Beer Sheva 84105 Israel and *University of Kansas Medical Center, Department of Medicine, Division of Clinical Pharmacology, Kansas City, Kansas 66103, U.S.A.

1 The α_2-adrenoceptor agonist, clonidine, reduces the hepatobiliary clearance of the anionic dye, sulphobromophthalein (BSP) in rodents. We now compare the effects of clonidine on BSP elimination with its effects on disposition of compounds which are metabolized by hepatic microsomal mixed function oxidases.

2 BSP, 100 mg kg^{-1} was administered i.v. to rats at 4 h after s.c. saline or clonidine, 0.2 mg kg^{-1}. Thirty min later, plasma BSP levels were 121.4 ± 2.25 μg ml^{-1} in saline-treated rats, while in clonidine-treated rats they were 631.5 ± 141.0 μg ml^{-1}. Clonidine raised hepatic BSP levels from 256.0 ± 28.9 μg g^{-1} tissue to 568.5 ± 86.5 μg g^{-1}.

3 Acute administration of clonidine (0.2 mg kg^{-1} s.c.) or repeated clonidine dosing (0.2 mg kg^{-1}, s.c. twice daily for 10 days) did not affect the disposition of intravenously administered [^{14}C]-antipyrine (15 mg kg^{-1}).

4 Activities of the P450 mixed function oxidase enzymes, aniline hydroxylase and aminopyrine N-demethylase, were identical in liver microsomes from saline-treated rats and in microsomes from rats given single or multiple s.c. doses of clonidine (0.2 mg kg^{-1}).

5 Addition of clonidine or other 2-substituted imidazoles at concentrations up to 2 μM did not affect the activities of aniline hydroxylase or of aminopyrine N-demethylase in suspensions of rat liver microsomes. Other substituted imidazoles, including cimetidine, clotrimazole and metronidazole, at concentrations of 0.2 μM or higher, inhibited the activities of these microsomal enzymes.

6 Clonidine slowed BSP elimination, which is probably hepatic blood flow-limited, but not the extraction-limited elimination of antipyrine, which is metabolized by hepatic microsomal enzymes.

Introduction

Clonidine (2-(2,6-dichloroanilino)-2-imidazoline) is an α_2-adrenoceptor agonist used clinically as an antihypertensive drug. Among its other activities, also mediated by adrenoceptors, are hypothermia, sedation, diuresis and various gastrointestinal effects. Clonidine is structurally related to other imidazole derivatives which have been shown to inhibit oxidative drug metabolism both *in vitro* and *in vivo*. Cimetidine, an H_2-receptor blocker which contains an imidazole moiety, slows the elimination of other drugs, including antiepileptic agents, benzodiazepines and theophylline (Sorkin & Darvey, 1983).

Recently we showed that clonidine inhibits the hepatobiliary elimination of the anionic dyes, sulphobromophthalein (BSP) and dibromosulphophthalein (DBSP) (Ben-Zvi & Hurwitz, 1985). These dyes are eliminated quickly by the liver and their elimination is mainly limited by the rate of hepatic blood flow. Since other imidazole-containing drugs slow the metabolism of extraction-limited drugs, we decided to assess the ability of clonidine to affect oxidative drug metabolism. Antipyrine elimination was chosen as a model for assessing clonidine effects on hepatic drug metabolism *in vivo*. Clonidine effects on the *in vitro* activities of microsomal mixed function oxidases were compared with other imidazole derivatives.

[1] Author for correspondence.

Methods

Male Holtzman-derived Sprague Dawley rats (Sasco Farms, Omaha, NE), weighing 300–350 g, were studied. They were housed in an environmentally controlled room and given water and rat chow *ad libitum*. On the day before each experiment, a cannula (PE 50) was placed in the right carotid artery under light ether anesthesia and exteriorized through the skin in the back of the neck. Cannula patency was maintained by flushing with heparinized saline (100 iu ml^{-1}). When given chronically, clonidine, 0.2 mg kg^{-1}, was administered s.c. twice daily for 10 days. In the acute experiment clonidine, 0.2 mg kg^{-1} was given s.c., a dose chosen following preliminary experiments. Thirty min after the last s.c. clonidine or saline dose, [^{14}C]-antipyrine was injected intraarterially at 15 mg kg^{-1} (containing 0.67 μCi mg^{-1}). The cannula was flushed after antipyrine administration. Arterial blood samples (0.2 ml) were obtained at 30 min intervals for 4 h after antipyrine. Blood levels of antipyrine were analysed radiometrically following extraction with chloroform according to Bakke *et al.* (1974). Pharmacokinetic parameters for antipyrine were calculated according to a one compartment open model.

When studying BSP disposition, this compound was injected at 100 mg kg^{-1} into arterial cannulae in rats 4 h after subcutaneous saline or clonidine, 0.2 mg kg^{-1}. The cannulae were flushed with heparinized saline. Thirty min after BSP, blood was withdrawn, the animals killed, and their livers quickly excised. Plasma BSP levels were measured spectrophotometrically after addition of 0.1 N NaOH, in a Gilford model 300N spectrophotometer at 580 nm. Hepatic BSP levels were determined after methanol extraction according to the method of Whelan & Combes (1971). The recovery of BSP from livers of rats was better than 94%. Rectal temperatures of the rats were measured before clonidine or saline administration and at the end of each experiment, with a Yellow Springs Thermometer, model ATC 73A.

In the *in vitro* experiments, rats were injected subcutaneously with saline or clonidine as described above. The rats were killed at 4.5 h after the last injection, livers removed quickly and microsomes prepared according to Cinti *et al.* (1972). Specific activities of aniline hydroxylase and aminopyrine N-demethylase were determined according to Kato & Gillette (1965) and Brodie & Axelrod (1950), respectively. Incubation conditions were as described by these authors. Microsomal protein was assayed by the method of Lowry *et al.* (1951). In experiments in which inhibitors were studied *in vitro*, these were added 10 min before substrate.

Clonidine and paraaminoclonidine were provided by Boehringer Ingelheim, Ltd. [N-methyl-^{14}C]-antipyrine of greater than 98.5% radiochemical purity was purchased from New England Nuclear, Boston, MA, U.S.A. Cimetidine and metronidazole were from Teva Pharmaceutical Industries, Israel. Clotrimazole was from Agis, Israel. Tolazoline was from Aldrich Chemical Co., Milwaukee, WI, U.S.A. All other chemicals were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Data were analyzed by one way analysis of variance (ANOVA) and Duncan's or Dunnett's test for statistically significant differences between saline and drug treatments.

Results

Clonidine impaired the hepatobiliary elimination of BSP (Table 1). Rats were given intraarterial doses of BSP, 100 mg kg^{-1}, 4 h after subcutaneous saline or clonidine, 0.2 mg kg^{-1}. Thirty min after BSP administration, body temperatures were lowered in clonidine-treated rats and levels of BSP in their plasma and livers were elevated. In contrast to its effect on BSP disposition, acute or chronic treatment with clonidine had no effect on antipyrine blood concentrations (Figure 1) or on the pharmacokinetic parameters of antipyrine disposition, including half

Table 1 Clonidine effects on sulphobromophthalein (BSP) disposition and body temperature in the rat

	Saline	*Clonidine*
Plasma BSP (μg ml^{-1})	121.4 ± 22.5 (11)	631.5 ± 141.0 (11)*
Hepatic BSP (μg g^{-1} tissue)	256.0 ± 28.9 (12)	568.5 ± 86.6 (11)*
Reduction in body temperature (°C)	0.54 ± 0.28 (9)	1.69 ± 0.31 (11)*

Rats were treated s.c. with saline or clonidine 0.2 mg kg^{-1}; 4 h later BSP was injected intraarterially. Blood samples were taken 30 min after BSP administration, rats were killed and liver samples removed. Temperatures were recorded before saline or clonidine and before blood sampling.
Number of animals at each point indicated in parentheses. Data are means ± s.e. mean. * $P < 0.05$.

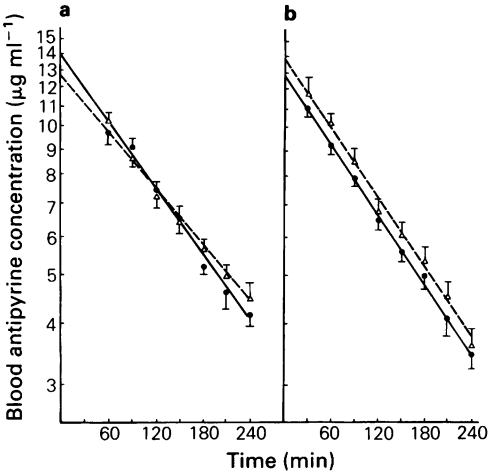

Figure 1 Effect of clonidine on antipyrine disposition. [^{14}C]-antipyrine (15 mg kg^{-1}) was injected intraarterially 30 min after a single s.c. dose of clonidine (0.2 mg kg^{-1}) (a) or chronic treatment with clonidine (0.2 mg kg^{-1} twice daily × 10 days) (b). Whole blood [^{14}C]-antipyrine was determined at indicated times. Data are means with s.e. mean shown by vertical lines; (●) saline; (△) clonidine.

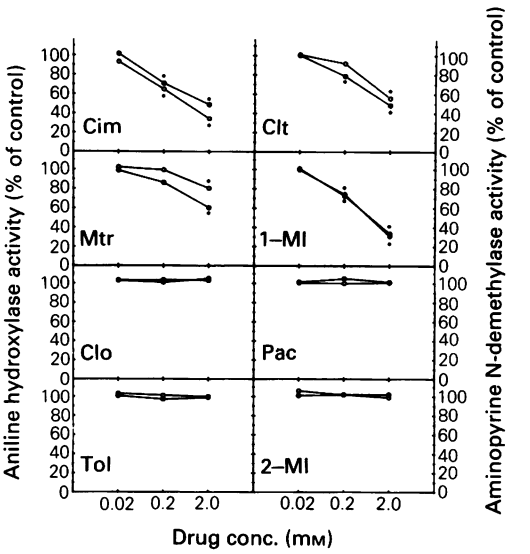

Figure 2 Effects of drugs containing the imidazole moiety on specific activities of hepatic aniline hydroxylase and aminopyrine N-demethylase. Cimetidine (Cim), clotrimazole (Clt), metronidazole (Mtr), l-methylimidazole (1-MI), clonidine (Clo), paraaminoclonidine (Pac), tolazoline (Tol) and 2-methylimidazole (2-MI) were added to the incubation mixtures of aniline hydroxylase (○) and aminopyrine N-demythylase (●) at 0.02, 0.2 and 2 mM. Specific activity, expressed as nmol product formed mg^{-1} protein min^{-1}, was determined as described in Methods and related to activity in the absence of inhibitor. Data are means of assays of hepatic microsomal suspensions from six rats; s.e. mean which were less than 5% of the mean at all values, are not displayed.
* $P < 0.05$ compared to saline control (100%).

life, volume of distribution or clearance (Table 2). The specific activities of cytochrome P450-mediated mixed function oxidase enzymes, aniline hydroxylase and aminopyrine N-demethylase, were not altered in livers of rats given acute or chronic clonidine treatment (Table 3). Addition of several imidazoles, but not clonidine or other 2-substituted imidazoles (*p*-amino clonidine, tolazoline and 2-methylimidazole), inhibited the activities of these enzymes when added to suspensions of rat liver microsomes *in vitro* (Figure 2).

Discussion

Clonidine is an α_2-adrenoceptor agonist which acts as an antihypertensive agent by reducing adrenergic outflow from the central nervous system. Recently we found that clonidine inhibits hepatobiliary excretory function (Ben-Zvi & Hurwitz, 1985). Excretion of the anionic dyes, BSP and DBSP, was inhibited by clonidine, resulting in higher levels of these dyes in plasma and liver and in reduced elimination into

Table 2 Effects of clonidine on pharmacokinetic parameters of antipyrine in the rat

	Acute (a)		*Chronic (b)*	
	Saline (8)	*Clonidine (11)*	*Saline (6)*	*Clonidine (5)*
k_{el} 10 (min^{-1})	5.74 ± 0.28	5.77 ± 0.28	5.49 ± 0.19	5.31 ± 0.30
$t_{1/2}$ (min)	122.8 ± 6.1	124.7 ± 7.2	127.2 ± 4.6	132.8 ± 4.7
V_d (ml kg^{-1})	975 ± 12	967 ± 27	1174 ± 66	1101 ± 64
Cl (ml min^{-1} kg^{-1})	5.58 ± 0.25	5.56 ± 0.36	6.41 ± 0.33	5.86 ± 0.49

Data displayed in Figure 1 were analyzed. Numbers in parentheses are animals in each group. No significant differences shown.
Abbreviations used: k_{el} = elimination rate constant; $t_{1/2}$ = elimination half life; V_d = apparent volume of distribution; Cl = clearance.

Table 3 Specific activities of mixed function oxidase enzymes in livers from clonidine-treated rats

Experiment 1

	Acute treatment	
	Saline-treated	*Clonidine-treated*
Aniline hydroxylase	0.209 ± 0.007 (7)	0.239 ± 0.018 (8)
Aminopyrine N-demethylase	0.104 ± 0.012 (8)	0.101 ± 0.010 (8)

Saline or clonidine (0.2 mg kg^{-1}) given s.c. After 4.5 h rats were killed, livers removed, microsomes prepared and enzyme activities assayed.

Experiment 2

	Chronic treatment		
	Saline-treated	*Clonidine-treated*	*Saline + clonidine*
Aniline hydroxylase	0.217 ± 0.007 (8)	0.224 ± 0.007 (8)	0.215 ± 0.009 (8)
Aminopyrine N-demethylase	0.101 ± 0.008 (8)	0.093 ± 0.006 (8)	0.096 ± 0.008 (8)

Saline or clonidine (0.2 mg kg^{-1}) s.c. was given twice daily for 10 days; 4.5 h after the last injection, rats were killed, livers removed and microsomes prepared. Enzyme activities were assayed in liver microsomes from rats treated with saline and clonidine and from saline-treated rats with clonidine added to the incubation mixture (2 mM).
Enzyme activities are expressed as nmol product formed mg^{-1} protein min^{-1}. Results are given as means ± s.e. mean. (n) = number in each group.
There were no significant differences.

bile. Yohimbine, an α_2-adrenoceptor antagonist, blocked this activity (Ben-Zvi & Hurwitz, 1986). Other α_2-adrenoceptor agonists, including methyldopa, guanabenz and clonidine derivatives, had similar effects on disposition of these model dyes, whose hepatic elimination is probably blood flow-limited (Ben-Zvi & Hurwitz, 1987).

In the present study, we evaluated the effects of clonidine on the disposition of antipyrine, a drug used frequently as a model for *in vivo* hepatic oxidative capacity. We also studied the effects of clonidine on hepatic cytochrome P450-mediated microsomal mixed function oxidase enzymes *in vitro*. These studies were prompted by several reports of impaired drug metabolism *in vivo* or exaggerated pharmacological effect due to combined administration of a drug containing an imidazole moiety or of an α_2-adrenoceptor agonist with other drugs (Wilkinson *et al.*, 1972; 1974; Wilkinson & Hetnarski, 1974; Sotaniemi *et al.*, 1977; Gachalyi *et al.*, 1980; Niemegeers *et al.*, 1981; Sorkin & Darvey, 1983). We first showed that, in the rat, clonidine impairs hepatic elimination of BSP and causes hypothermia for at least 4.5 h (Table 1). Thus, pharmacological effects of clonidine persisted well beyond the time needed to demonstrate any effects on antipyrine disposition. Hypothermia and impaired dye disposition after clonidine lasted much longer in rats than in mice (Ben-Zvi & Hurwitz, 1985).

Methyldopa is an antihypertensive agent (without an imidazole moiety) which is metabolized to an α_2-adrenoceptor agonist, methylnoradrenaline. This drug slows BSP elimination (Ben-Zvi & Hurwitz, 1987) and has potent effects on hepatic function (Dybing *et al.*, 1976; Sotaniemi *et al.*, 1977; Gachalyi *et al.*, 1980). It inhibits cytochrome P450-mediated mixed function oxidase *in vitro* (N-demethylase) and inhibits elimination of antipyrine and tolbutamide in man (Gachalyi *et al.*, 1980). The mechanism of this inhibition is as yet unknown, but may be related to the toxic effect of methyldopa on the liver and not to the effect mediated by α_2-adrenoceptors.

Many imidazole derivatives inhibit hepatic cytochrome P450-mediated oxidation both *in vivo* and *in vitro*. Potent inhibitors include cimetidine (Sorkin & Darvey, 1983), which is substituted at position 4 of the imidazole nucleus, 1 and 4-methylimidazole (Back & Tjia, 1985) and antimycotic drugs like miconazole and ketoconazole, substituted at position 1 (Niemegeers *et al.*, 1981), or benzimidazole and naphthimidazole compounds (James & Little, 1983). Results from the present study confirm previous findings that imidazoles with substitution of carbon 2, like clonidine, paraaminoclonidine, 2-methylimidazole and tolazoline did not inhibit aniline hydroxylase and aminopyrine N-demethylase (Wilkinson *et al.*, 1972; Back & Tjia, 1985; Kapetanović & Kupferberg, 1985). Another possible reason for the absence of effect of clonidine on antipyrine disposition and on oxidative enzymes is the low dose of clonidine used in the present study and therapeutically in man. Although this dose of clonidine

caused pronounced and prolonged hypothermia and impairment of BSP elimination, the relative content of the imidazole moiety in 0.2 mg kg^{-1} of clonidine may have been insufficient when compared to the high doses of imidazole derivatives used in studies which demonstrated inhibition of metabolism *in vivo*. Cimetidine, antimycotic drugs and methyldopa all inhibited hepatic function when given at therapeutic doses which are several orders of magnitude higher than that of clonidine.

Duration of hexobarbitone-induced loss of righting reflex is an accepted method for measuring hepatic oxidative drug metabolism *in vivo* in rodents. Timmermans *et al.* (1983) showed that clonidine prolonged hexobarbitone sleeping time in mice. However, clonidine has also been shown to prolong the hypnotic activities of halothane and chloral hydrate (Bloor & Flacke, 1982). Prolongation of hexobarbitone sleeping time by clonidine may be due to its central sedative action (Timmermans *et al.*, 1983) and thus does not prove activity on hepatic drug metabolizing enzymes, which would contradict our observations.

Our data suggest that clonidine would not affect oxidative drug metabolism in man since doses used clinically are much lower than those found to lack such effects *in vitro* and *in vivo* in rats. We therefore conclude that, when administered to rats at a fully effective pharmacological dose, clonidine raises plasma and liver levels of BSP, a dye whose elimination is believed to be hepatic blood flow-limited, but does not affect hepatic disposition of an extraction-limited drug, such as antipyrine.

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