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SHORT COMMUNICATIONS

The influence of chloracizine (Ch) and ftoracizine (Ft) on hexobarbital sleeping time, ethylmorphine *N*-demethylation and cytochrome P450 and b_5 concentration in rat liver

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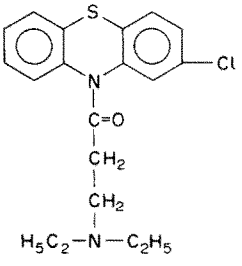
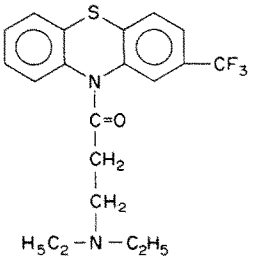
Phenothiazines are known to be inducers of liver microsomal monooxygenases [1–6], and chlorpromazine especially has been investigated by various groups. Moreover, chlorpromazine acts as a competitive inhibitor of cytochrome P450-dependent reactions [7, 8].

For new phenothiazines it is important to know whether they act as (acute) inhibitors or (chronic) inducers, especially if they are administered chronically for clinical purposes. This question is most important for the phenothiazines we investigated, since they have been introduced in the Soviet Union as spasmolytics and antidepressants for the treatment of endogenous depression. Moreover, these substances have some structural relationships to SKF 525 A, as can be seen from the following formulas:



Chloracizine (Ch)



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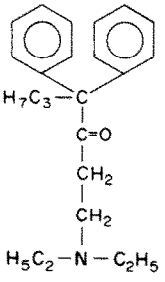

SKF 525 A

It has been demonstrated that the compounds chloracizine and ftoracizine inhibit hexobarbital elimination [9, 10]. Ch and Ft have been demonstrated to persist a long time in liver after a single injection [11, 12]. This paper shows that Ch and Ft act as inhibitors and inducers of drug metabolism.

MATERIALS AND METHODS

Colony-bred male, adult Wistar rats (aged about 6 months, weight 240–400 g) were used, bred and housed under standardized conditions [13]. The rats were i.p. injected with 50 mg/kg Ch or Ft, dissolved in water (10 ml/kg), once or three times. The control rats received the same volume of water.

Hexobarbital sleeping time was determined after i.p. injection of 100 mg/kg hexobarbital-Na [14]. Hexobarbital concentration in serum was determined according to Cooper and Brodie and according to Remmer, as described earlier [15]. Ch and Ft do not interfere with hexobarbital determination. For ethylmorphine *N*-demethylation *in vitro*, liver homogenate was used as enzyme source. Further details of incubation medium were described earlier [16].

RESULTS AND DISCUSSION

Thirty min after i.p. administration of 50 mg/kg Ch or Ft hexobarbital sleeping time and hexobarbital concentration in serum at awaking were increased (Table 1). This inhibitory action of Ch and Ft on drug metabolism can be demonstrated *in vitro* (Fig. 1).

In further experiments Ch or Ft were administered for 3 days (50 mg/kg daily), 1 day after the last injection, Ch and Ft prolong hexobarbital sleeping time and enhance hexobarbital concentration at awaking. Cytochrome P450 concentration and the rate of ethylmorphine *N*-demethylation also increased. Two days after the last Ch administration hexobarbital sleeping time is again within the normal range, ethylmorphine *N*-demethylation velocity and cytochrome P450 concentration are the same as 1 day before (Table 1). Neither 24 nor 48 hr after Ch administration is cytochrome b_5 content altered (not shown).

After single administration *in vivo* and *in vitro*, Ch and Ft proved to be strong inhibitors of ethylmorphine *N*-demethylation and hexobarbital elimination, i.e. of biotransformation.

After pretreatment for 3 days with Ch and Ft, cytochrome P450 concentration and ethylmorphine *N*-demethylation velocity were increased 1 and 2 days after the last injection. This can be interpreted as an induction effect. The lack of response of cytochrome b_5 was to be expected after the experiences with barbiturates as inducers [18]. But both substances prolonged hexobarbital sleeping time—this could be an additive effect on CNS action of hexobarbital or inhibition of hexobarbital breakdown in spite of the enhanced cytochrome P450 concentration and ethylmorphine *N*-demethylation rate. This result supports the earlier findings on a long persistence of these compounds in the body [11, 12]. The question, whether the CNS action or the inhibition of biotransformation is the reason for this prolongation of hexobarbital sleeping time cannot be solved by determination of *in vitro* ethylmorphine *N*-demethylation, since the homogenate is diluted 20-fold, and thus the actual inhibitor concentration

Table 1

		Control	(n)	Chloracizine	(n)	Ftoracizine	(n)
HBST (min)	30 min	42 ± 7	(9)	88 ± 4	(10)	93 ± 8*	(10)
	24 hr	42 ± 4	(9)	165 ± 20*	(6)	149 ± 13*	(6)
	48 hr	35 ± 2	(6)	37 ± 3	(6)	50 ± 3*	(6)
HBA (μg/ml)	30 min	20.7 ± 2.2		28.9 ± 3.0*		34.5 ± 5.3*	
	24 hr	27.0 ± 1.1	(10)	33.0 ± 1.4*	(6)	36.0 ± 1.5*	(6)
Cytochrome P450	24 hr	0.58 ± 0.02	(11)	0.91 ± 0.09*	(6)	0.87 ± 0.06*	(6)
(nmole/mg protein)	48 hr	0.54 ± 0.02	(11)	0.94 ± 0.05*	(6)	1.05 ± 0.05*	(6)
EMD(μmoleHCOH/g	24 hr	34.92 ± 5.67	(6)	59.22 ± 8.16*	(6)	52.78 ± 6.00*	(6)
protein × 20 min)	48 hr	31.65 ± 2.72	(6)	58.76 ± 4.12*	(6)	59.75 ± 2.66*	(6)

Influence of Ch and Ft on hexobarbital sleeping time (HBST), hexobarbital concentration in serum at awaking (HBA), cytochrome P450 concentration (cyt P450) in liver microsomes and on ethylmorphine *N*-demethylation (EMD) by liver homogenate 24 and 48 hr after the last administration. Ch or Ft were administered i.p. for 3 days (50 mg/kg daily). To demonstrate acute inhibitory effects of Ch and Ft, HBST and HBA were determined, also 30 min after a single i.p. administration of 50 mg/kg Ch or Ft. Arithmetic means + S.E.M. are given, number of determinations are in brackets. Statistically significant differences to controls ('*t*' test, $P \leqslant 0.05$) are marked by *

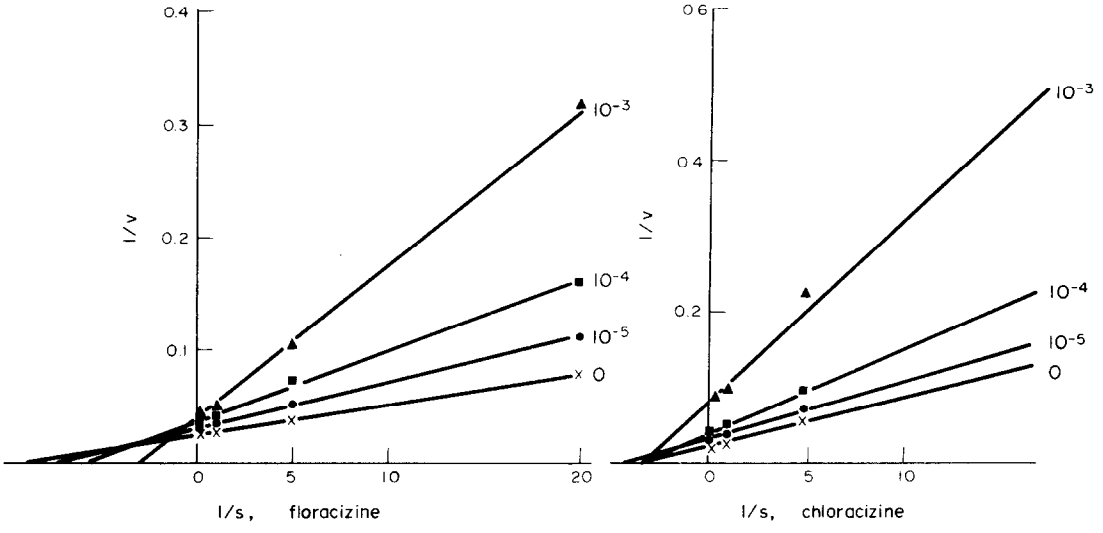

Fig. 1. Influence of Ch and Ft at concentrations of 10^{-3}, 10^{-4} and 10^{-5} M on ethylmorphine *N*-demethylation at substrate concentrations of 0.04–5 mM. Lineweaver–Burk plots of typical experiments, which have been reproduced three times with nearly identical results, are demonstrated.

is diminished. Thus we cannot observe the inhibition but only the induction of ethylmorphine *N*-demethylation. Two days after the last injection the effect of Ch and Ft on hexobarbital sleeping time has nearly completely disappeared. Ethylmorphine *N*-demethylation is the same as on the first day. As we ought to expect a decrease of hexobarbital sleeping time by induction, we must conclude, that small amounts of Ch and Ft are still present and counteract the decrease in hexobarbital sleeping time.

The strong inhibition effect of both compounds on hexobarbital biotransformation *in vivo* is supported by the determination of hexobarbital concentration at awaking. In spite of the longer time interval between hexobarbital administration and determination of hexobarbital concentration in the serum of Ch- or Ft-treated rats (because of longer sleeping time), hexobarbital concentration in these rats is higher than in control rats. However, if we assumed an effect of Ch and Ft on hexobarbital metabolism only, hexobarbital concentration at awaking would have to be the same as in control rats. The phenomenon of increased hexobarbital concentration at awaking must be explained as the development of acute tolerance in CNS.

In comparison to the results of Breyer [6] we can say that the induction effect of Ch and Ft is as potent as that of chlorpromazine.

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Desmethylimipramine-induced decrease in β-adrenergic receptor binding in rat cerebral cortex*

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Dibenzazepine derivatives like imipramine and desmethylimipramine are widely used in the treatment of depression. Their mechanism of action remains unknown. In 1964, Glowinski and Axelrod [1] reported that a single injection of these drugs to rats inhibits the uptake of intraventricularly administered radioactive norepinephrine (NE) into brain tissue. Inhibition of NE uptake could account for the reported prolongation and potentiation of adrenergic function produced by the tricyclic antidepressants [2] and is one of the cornerstones of the pharmacologically derived biogenic amine hypotheses of affective disorders [3–5].

Less is known about the effects of the tricyclic drugs when administered chronically. This is important because of the widely held (but not definitely proven) belief that there is a lag period before the tricyclic drugs exert an antidepressant effect [6]. It is of interest, then, that chronic administration of tricyclic drugs reduces some responses elicited by NE. For example, whereas a single injection of imipramine or desmethylimipramine has no effect on the ability of NE to elevate levels of endogenous adenosine 3′,5′-mono-phosphate (cyclic AMP) or [^{3}H]cyclic AMP net synthesis in slices of rat cerebral cortex [7], injection of these compounds for 5 days or longer was associated with a diminished response of the cyclic nucleotide to NE [7–10].

We have speculated previously [8] that the reduction in adrenergic responsiveness owing to repeated tricyclic drug administration may result, in part, from a decrease in adrenergic receptor sensitivity. In the present report, data consistent with this view are presented showing that the administration of desmethylimipramine produces a reduction of the binding of (−)[^{3}H]dihydroalprenolol to brain homogenates. (−)[^{3}H]dihydroalprenolol has been used to study β-adrenergic receptor binding sites in a number of tissues including brain [11–14].

Male Sprague–Dawley rats (225–300 g) were used in these experiments. Drug-treated animals were injected intraperitoneally each time with 10 mg/kg of desmethylimipramine hydrochloride (DMI; obtained as a gift from USV Pharmaceutical Corp.), while control animals received 0.9% NaCl. When two or more injections of DMI were administered, the injections were given twice each day. Except in the experiment in which animals were killed 1 hr after a single drug injection, all animals were killed by decapitation 24 hr after the final drug injection. After decapitation, the cerebral cortex was removed from the rest of the brain. Homogenization of this brain area and estimation of the extent of binding of (−)[^{3}H]dihydroalprenolol (New England Nuclear, Boston, MA; 32.6 Ci/m-mole) were done by modifications of the techniques described for brain by Alexander *et al.* [12] and Bylund and Snyder [14]. Like these authors, we also found that the binding of (−)[^{3}H]dihydroalprenolol to homogenates of cerebral cortex was saturable, reached equilibrium by 10 min, and was readily reversible by the addition of (±)propranolol. Specific binding of (−)[^{3}H]dihydroalprenolol (9 nM) to β-adrenergic receptors was assessed by the addition of (±)propranolol (1 μM) to the incubation media. All incubations, i.e. minus and plus propranolol, were done in quadruplicate. Specific binding averaged 50.0 ± 0.8 per cent ($\bar{x}$ ± S.E.M.). Filter blanks averaged less than 0.1 per cent of added counts.

The following procedure was used to estimate the concentration of DMI in cerebral cortex. After removal of the brain area, the tissue was dipped four times in an isotonic sucrose solution, blotted, and weighed. The tissue was then homogenized in 2 ml NaOH (0.1 M). An aliquot of the homogenate was diluted 1:40 with NaOH (0.1 M) and shaken in a glass-stoppered centrifuge tube with 5 vol of heptane containing isoamyl-alcohol (1.5%). The tubes

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