tions of ethacrynic acid. A significant decrease in titratable SH groups has been demonstrated with 1×10^{-4} M ethacrynic acid in the rabbit aorta (Needleman et al 1973) and thus it is likely that 2.5×10^{-4} M ethacrynic acid will have similar effects on trachealis muscle. In view of this it is probable that alkylation of sulphydryl groups is responsible for the interactions reported in this study. Needleman et al (1973) suggested that sodium nitroprusside and glyceryl trinitrate acted on primary receptors involving SH groups as well as the 'common intermediate site' also affected by isoprenaline. This may explain the differences in the effects of ethacrynic acid on the responses of the trachealis to isoprenaline on the one hand and glyceryl trinitrate and sodium nitroprusside on the other.

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Prolongation of thiopentone-induced sleep by trazodone and its metabolite, *m*-chlorophenylpiperazine

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Trazodone and its metabolite, *m*-chlorophenylpiperazine (CPP) prolonged significantly thiopentone-induced sleep in mice. Neither trazodone, nor CPP changed the cerebral concentrations of thiopentone. As cyproheptadine by itself did not affect thiopentone sleep and did not antagonize the effect of CPP, the effect of trazodone and CPP seems to be independent of their respective 5-HT-antagonistic and 5-HT-agonistic properties.

Interaction of drugs with barbiturates, measured as their influence on barbiturate sleeping time, is an old and commonly used test which is supposed to detect a general depressant action of a drug. However, the precise nature of prolongation of barbiturate sleeping time may differ from one drug to another, and may often be unknown. It may depend both on the kind of barbiturate and the mechanism of action of the tested drug. Thus, tricyclic antidepressants prolong the barbiturate sleeping time (see Gyermek 1966), but the interaction of these drugs with barbitone was ascribed to an increased CNS sensitivity to barbiturates, while the prolongation of pentobarbitone sleep was considered to result from inhibition of the hepatic metabolism of the barbiturate (Liu et al 1975). More recently, prolongation of thiopentone sleeping time by desipramine was ascribed to an interaction of desipramine with the noradrenergic system (Mason & Angel 1983).

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Trazodone, an atypical antidepressant drug (Silvestrini 1982), also prolongs barbiturate sleep (Silvestrini et al 1968), but the mechanism of this action remains unknown. The pharmacological profile of trazodone is dissimilar from that of other antidepressants (Maj 1978; Silvestrini 1982) and the drug seems to be devoid of a direct influence on the noradrenergic system but to interact in a complex manner with the brain 5-hydroxytryptamine (5-HT) system (Maj et al 1979). The complexity is caused by the fact that trazodone, which has central and peripheral anti-5-HT properties (Baran et al 1979; Maj et al 1979; Hingten et al 1984), is biotransformed with the formation of *m*-chlorophenylpiperazine (CPP) (Melzacka et al 1979; Caccia et al 1981) which has 5-HT-mimetic properties (Samanin et al 1979; Rokosz-Pelc et al 1980; Fuller et al 1981). We aimed to assess if trazodone and CPP have a similar effect on thiopentone sleeping time, if this effect is related to their 5-HT-antagonistic or 5-HT-mimetic properties, and if the two drugs change the cerebral levels of the barbiturate.

MATERIALS AND METHODS

Male mice of the CD-1, Albino-Swiss or C57BL/6 strain were kept under standard animal room conditions, with free access to food and water, for at least one week before the experiment.

Thiopentone sodium (Abbott), 50 mg kg^{-1}, was given 30 min after 0·9% NaCl (saline) solution or after trazodone HCl (Angelini), CPP HCl (synthesized in the Institute of Pharmacology, PAN, Krakow) or cyproheptadine HCl (Merck). All injections were given intraperitoneally in a volume of 10 ml kg^{-1}. Determinations of sleeping time always started between 9 and 10 a.m. Soon after thiopentone administration mice were put in plastic cages (30 × 12 × 12 cm; one mouse per cage), maintained at an ambient temperature of 22–23 °C. Sleeping time was measured as the time interval between loss and recovery of the righting reflex. A mouse was considered awake upon righting three times in 30 s.

In separate experiments the levels of thiopentone or trazodone and CPP were assayed in whole brain. Thiopentone was assayed with the spectrophotometric method of Goldbaum (1952) in brains of mice that had received the barbiturate 30 min after CPP or 60 min after trazodone injection; the mice were killed at various intervals after thiopentone administration. Brain levels of trazodone and/or CPP were assayed 15 or 60 min after the injection of the drugs. Trazodone was assayed with a spectrofluorometric method of Catanese & Lisciani (1970), and CPP—with the gas-chromatographic method of Rurak & Melzacka (1983).

The doses of trazodone and CPP were expressed in μmol kg^{-1} (rounded to the nearest μmol) to allow comparison of their potency.

Results and discussion

Both trazodone and CPP prolonged the thiopentone sleeping time (Fig. 1). As equivalent doses of CPP acted more strongly than those of trazodone, it appears that the potentiation of thiopentone action by trazodone may be caused, or at least assisted, by CPP formed from the parent compound.

To investigate this we measured the brain levels of CPP and trazodone after administration of the compounds. The results (Table 1) indicate that CPP accumulated rapidly in the mouse brain, and was rapidly eliminated. In fact, after the dose of 4μmol kg^{-1} the CPP cerebral concentration after 1 h was close to the detection limits; this dose did not prolong thiopentone sleeping time. After administration of trazodone, the level of the parent compound decreased between 15 and 60 min after the injection, but considerable concentrations of CPP were present in the brain, particularly at 60 min.

As some drugs prolong the barbiturate sleeping time because of impeding of barbiturate metabolism (e.g. Liu et al 1975), we tested if the action of trazodone and CPP could be explained by such a phenomenon. Neither trazodone (Table 2) nor CPP (Fig. 2) significantly affected the cerebral level of thiopentone.

As both trazodone and CPP affect 5-HT-ergic system (Maj et al 1979; Baran et al 1979; Samanin et al 1979; Rokosz-Pelc et al 1980) it was tempting to discuss the present findings in terms of the interactions of barbiturates with 5-HT mechanisms. However, the relation between barbiturates and 5-HT system has not been much studied, and a review of the mode of action of

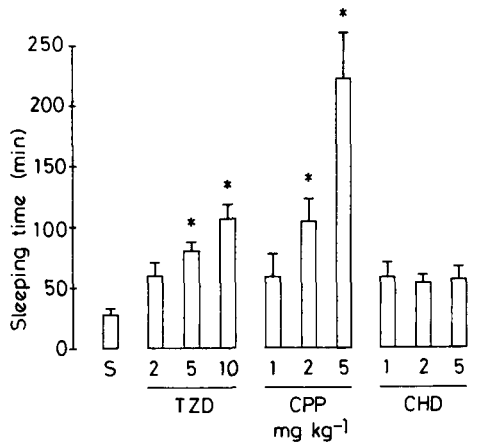

FIG. 1. Sleeping time induced by thiopentone sodium (50 mg kg^{-1}), given 30 min after saline (S), trazodone (TZD), CPP or cyproheptadine (CHD) in CD-1 mice. The data are means ± s.e.m. The number of animals per group was: S—30, TZD—20, CPP—10 and CHD—10, for each dose. Asterisks indicate a significant difference (Student's t-test; $P < 0.001$) from control (S).

Table 1. Brain CPP and trazodone concentrations after administration of the compounds in Albino-Swiss mice.

Compound, dose (μmol kg^{-1})	CPP concn (nmol g^{-1}) 15 min	60 min	Trazodone concn (nmol g^{-1}) 15 min	60 min
CPP, 4	6·4 ± 1·4	0·2 ± 0·1	N.T.	N.T.
CPP, 9	16·9 ± 2·9	6·5 ± 0·5	N.T.	N.T.
Trazodone, 24	N.T.	6·1 ± 0·1	N.T.	1·1 ± 0·1
Trazodone, 48	8·1 ± 0·6	21·7 ± 3·2	6·4 ± 0·9	1·8 ± 0·2

N.T. = Not tested. Each group consisted of 5 mice. The data are means ± s.e.m. The concentrations are calculated for fresh tissue weight. The exact doses of drugs were: CPP 1 and 2 mg kg^{-1}; trazodone 10 and 20 mg kg^{-1}.

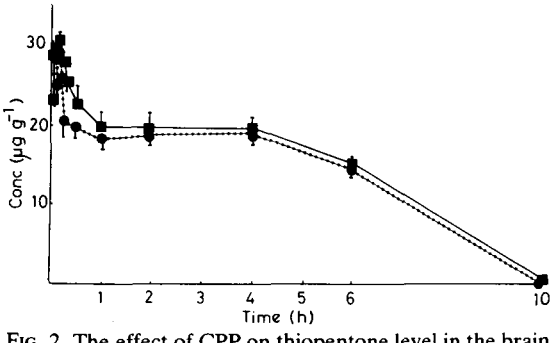

FIG. 2. The effect of CPP on thiopentone level in the brain of Albino-Swiss mice. Thiopentone (TP), 50 mg kg^{-1}, was given 30 min after injection of CPP, 9 μmol kg^{-1} (2 mg kg^{-1})a i.p. The TP concentrations in the whole brain are given in μg g^{-1} of the fresh tissue. Each point represents the mean (± s.e.m.) from 8 experiments.

Table 2. The effect of trazodone on cerebral thiopentone level in Albino-Swiss mice.

Time after thiopentone inj. (min)	Cerebral thiopentone level ($\mu g\ g^{-1}$)	
	Saline pretreatment	Trazodone pretreatment
15	28·2 ± 1·1	29·7 ± 2·5
30	21·9 ± 2·7	24·5 ± 1·2
60	21·3 ± 2·5	21·4 ± 2·2

Each group consisted of 3 animals. The data are means ± s.e.m. and are calculated for fresh tissue.
Thiopentone, 50 mg kg^{-1}, was given 1 h before saline or trazodone, 24 μmol kg^{-1}.

barbiturates ignores the interaction between barbiturates and 5-HT transmission (Ho & Harris 1981). A review of older studies (Nicoll 1978) quotes the results indicating that the inhibitory 5-HT transmission is not affected by barbiturates, which may, however, antagonize the excitatory effects of various neurotransmitters.

To find if the 5-HT-ergic system might be involved in the action of CPP, we investigated the effect of a 5-HT-antagonist, cyproheptadine (van Riezen 1972), tested alone or with CPP on thiopentone sleeping time. The results show that the 5-HT-antagonist by itself did not affect the sleeping time (Fig. 1), and the thiopentone-sleeping time in mice receiving cyproheptadine and CPP was even longer than in the animals receiving either of drugs alone (Table 3). A similar effect was observed in another strain of mice (Albino-Swiss: data not shown). This suggests that 5-HT-ergic mechanisms are not crucially involved in thiopentone sleep and in the prolongation of sleep by CPP.

The present findings indicate therefore that the prolongation of thiopentone sleep by trazodone is probably caused or assisted by formation of CPP, and that the action of trazodone and CPP is not caused by changes in thiopentone metabolism and is not related to 5-HT-mimetic properties of CPP. Thus, the present results indicate, as did our studies on CPP action on avoidance behaviour (Sansone et al 1983; Vetulani et al 1984), that some pharmacological effects of trazodone and CPP cannot be interpreted in terms of their actions on 5-HT-system.

Table 3. The effect of CPP and cyproheptadine on thiopentone sleeping time. Mice of the C57BL/6 strain received thiopentone 50 mg kg^{-1}, 30 min after CPP and cyproheptadine, given alone or in combination. The data are means ± s.e.m. from 6 animals.

Treatment, dose (μmol kg^{-1} i.p.)	Sleeping time (min)
Saline	81·5 ± 17·8
CPP, 9	162·5 ± 26·1*
Cyproheptadine, 15	102·3 ± 5·1
Cyproheptadine, 15 + CPP, 9	270·0 ± 40·6**

Significant difference from saline control (Student's *t*-test): $^*P < 0.05$; $^{**}P < 0.01$.

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