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Effect of optical isomers of (±)-fenfluramine on pentobarbitone brain and plasma concentrations and sleeping time

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Many drugs affect barbiturate sleeping time in various animal species by acting on liver metabolism of the hypnotic and/or on the central nervous system (Gaudette & Brodie 1959; Axelrod et al 1964; Straw et al 1965; Conney et al 1967; Shah & Lal 1971; Beaubien et al 1976). For instance chlorpromazine increases sleeping time and pentobarbitone brain concentrations by inhibiting barbiturate metabolism both in vivo and in vitro (Jori et al 1970), while amphetamine stimulation of c.n.s. activity reduces sleeping time without affecting barbiturate brain concentrations (Kato et al 1964). In contrast to amphetamine, fenfluramine potentiates barbiturate narcosis as shown by Le Douarec et al (1966).

We investigated the effects of various doses of fenfluramine and of different intervals between fenfluramine and pentobarbitone administration and compared the (+)- and (−)-isomers of fenfluramine on sleeping time and pentobarbitone plasma and brain concentrations in male rats. A sensitive gas-chromatographic assay for pentobarbitone in tissues has been developed.

Materials and methods

Chemicals. (±)-Fenfluramine HCl and its (+)- and (−)-optical isomers were obtained from Laboratories Servier, Orléans, France. Sodium pentobarbitone and sodium secobarbital (quinalbarbitone) were supplied by Abbott Laboratories (Chicago, U.S.A.); the latter was dissolved in 0·9% NaCl (saline) immediately before use. All other chemicals were of reagent grade.

Animals. Male CD-COBS rats (Charles River, Italy), 220–250 g, were housed under controlled conditions of 22 ± 0·5 °C, 60 ± 5% humidity and 12 h light 12 h darkness (7 am–7 pm; 7 pm–7 am). The animals had free access to food (Altromin, MT, Rieper, Italy) and water.

Sleeping time experiments. Rats were injected intraperitoneally with sodium pentobarbitone (30 mg kg^{-1}) in saline. Sleeping times were measured to the nearest half minute from the loss to the return of the righting reflex. All experiments on pentobarbitone sleeping time were between 9 and 10 am. Plasma and brain concentrations were determined 90 min after the barbiturate.

(±)-Fenfluramine or its analogues were injected intraperitoneally at different doses (3·12; 6·25; 12·5 and 25 mg kg^{-1}) and at different intervals (1, 3, 6, 12 and 24 h) before pentobarbitone treatment. Control rats received saline (5 ml kg^{-1}).

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Gas chromatographic determination of pentobarbitone in brain and plasma. Rats were decapitated 90 min after pentobarbitone treatment and the brain immediately removed, weighed and rapidly homogenized in distilled water (1:4 w/v) in an Omnimixer at 11 000 rev min^{-1} for 2 min at 0 °C. The homogenate (2 ml) was mixed with 1 M HCl (0·4 ml) and chloroform (10 ml); 20 µl of sodium secobarbital methanol solution (4 µg) was added as internal standard. Samples were shaken (100 oscillations min^{-1}) for 30 min at room temperature (20 °C) then centrifuged (2000 rev min^{-1} for 20 min at −10 °C). The chloroform layer was extracted with 1 M NaOH (4 ml) and centrifuged as above. Chloroform was then discarded, the aqueous phase was acidified with 6 M HCl (0·8 ml) and pentobarbitone re-extracted into fresh chloroform. After centrifugation the chloroform extract was evaporated to dryness in a waterbath at 75 °C.

Blood from the rats was collected in tubes containing Na-citrate (0·1 ml of 3·8 sol per ml^{-1} blood) and centrifuged (2000 rev min^{-1} for 10 min at 4 °C). Plasma (0·5 ml) was mixed with 1 M HCl (50 µl) and chloroform (4 ml); sodium secobarbital 50 µl was added as internal standard (10 µg). After centrifugation (2000 rev min^{-1} for 20 min at 4 °C) the chloroform layer was brought to dryness without further extractions as described for brain.

The dry brain and plasma residues were dissolved in methanol (25 µl) and 1 µl of the sample was mixed with 1 µl of trimethylanilinium hydroxide 0·2 M and injected into the gas chromatographic column (Brochmann-Hanssen & Oke 1969).

A gas chromatograph (Fractovap 2003, C. Erba) equipped with flame ionization detector was used: the glass column (3 m × 3 mm i.d.) packed with Gas-Chrom Q (100–120 mesh) coated with SE-30 5% was conditioned for 24 h at 290 °C before use. Operating conditions were: injector temperature 275 °C; column temperature 185 °C; nitrogen flow 25 ml min^{-1}; hydrogen flow 25 ml min^{-1} oxygen flow 250 ml min^{-1}.

Unknown pentobarbitone quantities were determined by comparing peak areas with those of the secobarbital standard. The total recovery of pentobarbitone from brain and plasma samples was respectively 75 ± 5% and 80 ± 4%. In several experiments pentobarbitone was measured using the flurorimetric method of Brodie et al (1953).

Dunnett's (1955) test was employed to compare the differences between various experimental groups and controls.

Table 1. Pentobarbitone sleeping time in animals pretreated with (±)-fenfluramine or isomers.

Treatment		Pentobarbitone sleeping time (min)
Control (a)		53 ± 15
(±)-Fenfluramine	25 mg kg^{-1}†	153 ± 11**
Control†		53 ± 6
(±)-Fenfluramine	12·5 mg kg^{-1} i.p.‡	80 ± 6*
(±)-Fenfluramine	6·25 mg kg^{-1} i.p.‡	64 ± 6
(±)-Fenfluramine	3·12 mg kg^{-1} i.p.‡	45 ± 3
Control†		54 ± 4
(+)-Fenfluramine	25 mg kg^{-1} i.p.‡	187 ± 22**
(−)-Fenfluramine	25 mg kg^{-1} i.p.‡	199 ± 23**
Control		63 ± 12
(+)-Fenfluramine	12·5 mg kg^{-1} i.p.‡	100 ± 13*
(−)-Fenfluramine	12·5 mg kg^{-1} i.p.‡	98 ± 10*

† Na pentobarbitone 30 mg kg^{-1} i.p.
‡ Fenfluramine was injected 1 h before the barbiturate.
Mean ± s.e. of five rats.
$P < 0.05$.
$P < 0.01$.

Results

In the groups of five rats treated with (±)-fenfluramine 60 min before pentobarbitone (30 mg kg^{-1} i.p.), barbiturate sleeping time was significantly longer than in controls when (±)-fenfluramine was given at the doses of 25 and 12·5 mg kg^{-1} i.p. (Table 1), but not at the lower doses.

Other experiments with the isomers at the doses of 25 and 12·5 mg kg^{-1} i.p. showed their ability to potentiate the sedative action of the barbiturate was similar to (±)-fenfluramine (Table 1).

When (±)-fenfluramine (25 mg kg^{-1} i.p.) was administered 1 to 12 h before pentobarbitone, sleeping time was significantly prolonged, but when the doses were given 24 h apart, the values were normal (Table 2).

Whole brain and plasma concentrations of pentobarbitone were assayed by gas-chromatography in control and (±)-fenfluramine-pretreated groups 90 min after barbiturate injection. Brodie's spectrofluorimetric method was used to assay whole brain values of pentobarbitone 60 min after administration in experiments where the doses of (±)-fenfluramine and barbiturate were given 3 and 12 h apart. In these conditions fenflurmine pretreatment 1 to 12 h before pentobarbitone enhanced brain and plasma barbiturate concentrations (Table 3).

Table 2. Effect on sleeping time of various intervals between (±)-fenfluramine and pentobarbitone administration.

Time between fenfluramine pretreatment (25 mg kg^{-1})i.p. and pentobarbitone administration (30 mg kg^{-1} i.p.) (h)	Pentobarbitone sleeping time (min) (mean ± s.e.n = 5)	
	Controls	Treated
1	39 ± 2·4	152 ± 13**
3	42 ± 2	125 ± 13**
6	34 ± 2	110 ± 7**
12	35 ± 2	75 ± 4**
24	45 ± 3	46 ± 6

** $P < 0.01$.

Table 3. Plasma and brain pentobarbitone concentrations in animals pretreated with (±)-fenfluramine (25 mg kg^{-1} i.p.) at different intervals. Mean ± s.e. of five assays.

Time between pretreatment and pentobarbitone (30 mg kg^{-1} i.p.) (h)	Brain (μg g^{-1})		Plasma (μg ml^{-1})	
	Controls	(±)-Fenfluramine treated	Controls	Treated
1 (g.c., f)	8 ± 1·5	16·5 ± 2·2**	6·0 ± 0·8	9·4 ± 1**
3 (f)	9 ± 1·2	17 ± 0·8**	—	—
6 (g.c., f)	9 ± 1	21 ± 1·5**	6·3 ± 1	10·3 ± 0·4*
12 (f)	11 ± 1	14·5 ± 1	—	—
24 (g.c.)	12 ± 2	12 ± 1	8 ± 1·5	9 ± 1

g.c. Gas-chromatographic determination 90 min after pentobarbitone.
f Fluorimetric determination 60 min after pentobarbitone.
** $P < 0.01$.

Discussion

Barbiturate sleeping time may be correlated with brain and plasma drug concentrations (Greig & Mayberry 1951; Kato et al 1969). Fenfluramine's effect on barbiturate narcosis is dose-related (Le Douarec et al 1966) but no correlation is reported of fenfluramine dose and the prolonged sleeping time with brain and plasma barbiturate concentrations. The present study has shown that fenfluramine's effect depends on the interval between its administration and pentobarbitone treatment and also that the racemate and isomers do not differ in their effect on barbiturate narcosis. Brain and plasma concentrations of pentobarbitone may be correlated with the length of sleeping time, but this still does not explain the possible interaction between the drugs. However it is known that the fenfluramine is largely metabolized in the liver so it may well compete with pentobarbitone for the microsomal oxidase system. The metabolism of barbiturate and fenfluramine are both inhibited by SKF-525-A (Cooper et al 1954; Jori et al 1978) perhaps the metabolic pathways of these two kinds of drugs develop using factors possessed in common. This suggests that fenfluramine increases pentobarbitone sleeping time mostly because of competitive inhibition of pentobarbitone metabolism which results in raised brain levels of the barbitone. This agrees with the results obtained by J. C. Le Douarec who found that fenfluramine increased the pentobarbitone sleeping time when it was administered before the barbiturate but not when it was administered after.

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The metabolism of sennosides A and B by the intestinal microflora: in vitro and in vivo studies on the rat and the mouse

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The glycoside laxatives sennosides A and B should be considered as inactive precursors in which the sugar moiety acts as a transport group (Fairbairn & Moss 1970). They are hydrolysed in the organism into their aglycones at least in part by the action of bacterial enzymes (Lemmens 1979) and these aglycones by influencing the water and electrolyte transport in the colon are responsible for the laxative action (Lemmens 1974, 1976).

The present report deals with the in vitro and in vivo metabolism by the rat and mouse microflora of sennosides A and B. The role of the bacteria in the metabolism of sennoside A has been established by a comparative study on conventional (CVL) and germ free (GF) animals.

Materials and methods

Compounds. Sennosides A and B were commercially available (C. Roth, Karlsruhe, Germany) chrom. depur. Sennidins A and B were prepared by hydrolysing the sennosides with sulphuric acid (Lemmens 1977). Rhein was prepared from aloin by the method of Bellaart (1952). Rhein-9-anthrone was prepared by reduction of rhein in acid medium according to Auterhoff & Scherff (1960). Rhein-1-monoglucoside was prepared according to Bellaart (1952).

The compounds were checked chromatographically for purity and purified if necessary.

In vitro incubation with rat caecal contents. All test solutions were protected from light. The test substance (0·5–1·5 mg) was dissolved in 0·5% sodium bicarbonate solution and sterilized by filtration (Millipore 0·2 μm). One ml of this solution was added to a test tube containing 10 ml of sterile Bacto Tryptone Yeast extract broth (ISP medium 1—Difco Laboratories, Michigan) in 0·1 M phosphate buffer pH 7·00.

Caeca were obtained from CVL or GF female Fisher rats. Both CVL and GF animals were maintained on a commercial pelleted diet (Hope Farms, The Netherlands) enriched with vitamins to compensate for loss during sterilization (autoclaving at 121 °C for 25 min). Caecal extracts of CVL rats were prepared by mixing the entire caecal contents with 10 ml of sterile phosphate buffer and centrifuging for about 1 min at low speed to remove debris. Caeca of GF rats were surgically removed in the GF isolator (Trexler plastic isolators, H. G. Kleinfeld, Hannover, Germany) and the watery contents collected in a sterile test tube. The tube was centrifuged and 1 ml of the supernatant was aseptically added to the test solution before incubation. Two control samples were prepared similarly except that either the caecal extract or the test compound was omitted.

The tubes were incubated anaerobically at 37 °C. Test samples were removed at regular intervals and were immediately frozen at −20 °C.

Extraction of incubates. After the incubates had thawed, the samples were examined quantitatively and qualitatively as follows: the contents of a test tube were extracted with three 10 ml portions of acidified, peroxide-free diisopropylether, to avoid oxidation of labile metabolites such as rhein-9-anthrone. The ether extracts were divided in two equal portions and evaporated to dryness under reduced pressure without heating. One portion of the evaporated extract was dissolved in 0·5 ml of a 0·1% solution of *p*-nitrosodimethylaniline in pyridine to form the azomethine derivative of rhein-9-anthrone which gives a blue green colour. This derivative is chromatographically more easily separated from rhein than rhein-9-anthrone itself. The second portion of the evaporated extract was dissolved in 0·5 ml ethanol for chromatography of the other aglycones. The remaining aqueous solution was used for quantitative determination (Lemli 1965) of the glycosides as a function of time. The total amount of anthracene derivatives present after incubation was quantitatively determined by the same method, but without previous extraction of the aglycones.

Chromatography. The pyridine and methanol solution, together with appropriate standards, were examined by thin layer chromatography on 0·25 mm thick layers of precoated silicagel 60 GF_{254} (Merck, Darmstadt, Ger-

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