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Changes in regional concentrations in the rat brain of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid during the development of tolerance to the sedative action of chlordiazepoxide

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Benzodiazepines cause sedation following acute administration (Greenblatt & Shader 1974). With continued treatment, however, a tolerance develops to the c.n.s depressant effect which cannot be attributed solely to pharmacokinetic changes (Greenblatt & Shader 1978). There have been several studies investigating the effects of acute and chronic benzodiazepine administration on brain 5-hydroxytryptamine (5-HT), but few follow the development of tolerance to benzodiazepines both behaviourally and biochemically in the same animals. We did this by measuring locomotor activity and exploration in a holeboard apparatus and measuring concentrations of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in several discrete brain regions in rats treated acutely or chronically with chlordiazepoxide (10 mg kg^{-1}), which has a profound sedative action to which animals become tolerant after 10 days (File 1982).

Methods

Animals. Male hooded-rats (Olac Ltd, Bicester) (400 g) were housed in groups of eight and allowed free access to food and water. They were kept in an 11 h light–13 h dark cycle (lights on 06.30).

Chlordiazepoxide hydrochloride (Roche Products Ltd) was dissolved in distilled water to give a solution of the free base of 5 mg ml^{-1}.

The holeboard was a wooden box 36 × 60 × 60 cm with four holes in the floor. Infra-red cells in the walls of the box and just below each hole provided automated measures of locomotor activity, rearing, the number of head-dips and the time spent head-dipping.

Procedure

Thirty-two rats were divided into four equal groups and injected (i.p.) with vehicle, or chlordiazepoxide (10 mg kg^{-1}), either acutely, for 5 days or for 10 days. Each cage contained an equal number of animals from each group.

On day 10, thirty min after the last injection each rat was placed alone in a holeboard apparatus (File & Wardill 1975) for a 7·5 min test. The animal was then decapitated, the brain removed and stored at −20 °C. Animals were tested between 07:30 and 12:00 h in an order randomized for drug group. Between each trial the box was cleaned. 5-HT and 5-HIAA concentrations in the frontal cortex, hypothalamus, hippocampus and cerebellum were determined using the fluorometric method of Curzon et al (1981). All assays were performed within 4 weeks. The behavioural data of the drug-treated groups were analysed using analysis of variance with the number of days of drug treatment as the independent factor. Comparisons between the control and acutely treated animals were made using Student's *t*-test.

The biochemical data for all groups were analysed using analysis of variance and between-group comparisons were made using Dunnett's multiple range test.

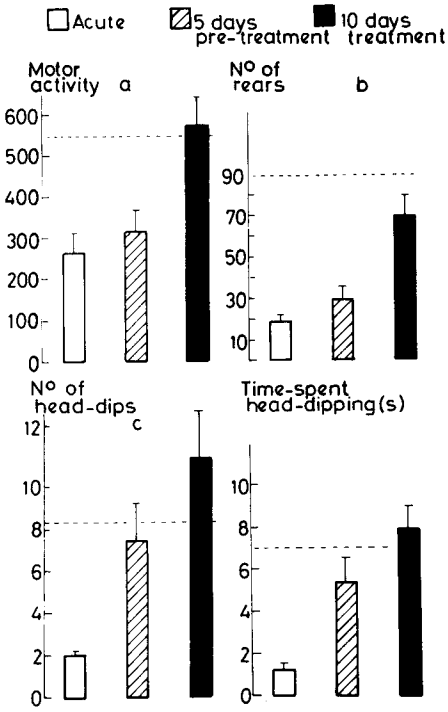

FIG. 1 Locomotor activities (a) the number of rears (b) (c) head-dips and the time spent head-dipping (d) during a 7·5 min trial in a holeboard apparatus. Rats received chlordiazepoxide (10 mg kg^{-1}) acutely (open columns), for 5 days (hatched columns) or for 10 days (shaded columns). The scores of vehicle treated animals are represented by the broken horizontal lines. Scores are means ± s.e.m.

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Results

Acute treatment with chlordiazepoxide caused a reduction in locomotor activity, rearing, the number of head-dips and the time spent head-dipping ($P < 0{\cdot}001$), reflecting the sedative action of the drug (see Fig. 1). Significant tolerance to chlordiazepoxide was shown for all behavioural tests i.e. animals had higher locomotor activities ($F(2, 21) = 8{\cdot}9$, $P < 0{\cdot}002$), reared on more occasions ($F(2, 21) = 14{\cdot}0$, $P < 0{\cdot}001$), head-dipped for longer ($F(2, 21) = 12{\cdot}5$, $P < 0{\cdot}001$) and more frequently ($F(2, 21) = 7{\cdot}5$, $P < 0{\cdot}005$) the longer the period of chlordiazepoxide pretreatment (see Fig. 1). The concentrations of 5-HT and 5-HIAA in the brain region studied are shown in Table 2. The only significant change was the increase in 5-HT concentration in hippocampus and frontal cortex ($P < 0{\cdot}025$, Dunnett's test) following acute administration of chlordiazepoxide.

Discussion

The increase in 5-HT concentration following acute treatment with chlordiazepoxide is consistent with previous results showing reduced 5-HT turnover after a single dose of a benzodiazepine (Wise et al 1972: Cook & Sepinwall 1975: Rastogi et al 1978: Pratt et al 1979); Dominic et al (1975) also showed a reduction in 5-HT synthesis after acute treatment with chlordiazepoxide 10 mg kg^{-1}. Our failure to find a reduction in 5-HIAA concentrations was probably a result of the reduced rate of disappearance of the compound from the brain (Chase et al 1970). After 5–10 days of pretreatment with chlordiazepoxide, 5-HT concentrations were no longer significantly elevated. Differences between the results of this study and one using 5 mg kg^{-1} chlordiazepoxide (File & Vellucci 1978) suggest that its effects on 5-HT synthesis and turnover vary with dose, and so tolerance to the synthesis and turnover may develop at different rates. Jenner et al (1975) found that increases in 5-HT and 5-HIAA following acute treatment with clonazepam were not present after eight days. Benzodiazepine receptor subsensitivity has been reported in animals following chronic treatment with clonazepam (Crawley et al 1982), and hepatic enzyme induction could also have contributed to the tolerance observed in the clonazepam study. However, neither changes in benzodiazepine receptor sensitivity nor hepatic enzyme induction account for the behavioural tolerance to the effects of chlordiazepoxide observed in this study (Crawley et al 1982; File 1982).

Table 1. Animals received i.p. injections of either vehicle (VEH) or chlordiazepoxide (CDP, 10mg kg^{-1}) on each day of the experiment.

	Drug treatment		
Group	Days 1–5	Days 6–9	Day 10
Control	VEH	VEH	VEH
Acute CDP	VEH	VEH	CDP
5 Days CDP	VEH	CDP	CDP
10 days CDP	CDP	CDP	CDP

Our results show that, before the 10th day of treatment, tolerance develops both to the sedative action of chlordiazepoxide (10 mg kg^{-1}) and to its effect of increasing 5-HT concentrations in the hippocampus and frontal cortex. Either 5-HT turnover must therefore have increased, or the rate of 5-HT synthesis must have decreased, during the period of treatment. So far the only reported change in 5-HT synthesis during chronic treatment has been an increase (Rastogi et al 1978).

Wise et al (1972) reported that tolerance developed to the reduction in noradrenaline but not 5-HT turnover, after 6 days of treatment with oxazepam (20 mg kg^{-1}). They therefore suggested that reduced noradrenaline and not 5-HT turnover was responsible for the sedative action of the benzodiazepine. They also reported that some tolerance did develop to the effect of oxazepam on 5-HT turnover. Cook & Sepinwall (1975) found no change in noradrenaline turnover after a single dose of chlordiazepoxide (10 mg kg^{-1}) and found some tolerance to the decrease in 5-HT turnover between the second and third days of treatment. Although a recent study found a reduction in 5-HT turnover to persist for 22 days during treatment with diazepam (10 mg kg^{-1}), concomitant continuing sedation was also reported (Rastogi et al 1978). Certainly, the link between 5-HT

Table 2. Levels of 5-HT and 5-HIAA (ng g^{-1}) in various brain regions of rats treated with vehicle, chlordiazepoxide (CDP 10 mg kg^{-1}) acutely, for 5 days or for 10 days. Figures are means ± s.e.m.

	Cerebellum		Frontal Cortex		Hippocampus		Hypothalamus	
	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA
Vehicle	156 ±10	204 ±24	519 ±19	746 ±19	574 ±25	722 ±28	980 ±46	1064 ±83
Acute CDP	174 ±14	209 ±13	600* ±17	745 ±36	667* ±29	711 ±42	1135 ±59	1086 ±93
5 days CDP	162 ±5	205 ±5	546 ±20	748 ±30	660 ±23	841 ±74	977 ±84	807 ±30
10 days CDP	154 ±10	190 ±8	559 ±29	762 ±16	614 ±27	740 ±71	1074 ±85	1027 ±38

* Significantly different from vehicle $P < 0{\cdot}025$, Dunnett's test.

and the sedative actions of the benzodiazepines seems to have been prematurely dismissed and further studies combining behaviour with biochemistry are needed to clarify the role of this neurotransmitter in benzodiazepine-induced sedation.

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Biological activity of indoprofen and its optical isomers

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Indoprofen, (α[4-(2-isoindolinyl-1-one)-phenyl] propionic acid) a non-steroidal analgesic anti-inflammatory drug (NSAID) (Buttinoni et al 1973; Bruni et al 1980) has an asymmetric carbon atom and can therefore occur either as the (+)- or (−)-isomer. The optically active indoprofen enantiomers have been resolved (Tosolini et al 1974) and their absolute configurations determined (De Munari et al 1980). Several workers have claimed that biological activity in some substituted phenylpropionic acids is due almost entirely to the (+)-isomer (Shen 1967; Ham et al 1972; Takeguchi & Sih 1972; Tomlinson et al 1972; Greig & Griffin 1975) believed to have the *S*-configuration (Wechter et al 1974; Simmonds et al 1980; Tamura et al 1981). The aim of this study was to determine the contribution of the enantiomers to the activity and toxicity of racemic indoprofen. We also investigated their effect on prostaglandin biosynthesis, as the biological activity of this class of compounds is closely linked to inhibition of the prostaglandin system.

Methods

Anti-inflammatory activity was studied in male ICEM:CER (SPF Caw) rats on acute and subchronic models, carrageenan oedema (Winter et al 1963) and granuloma pouch (Boris & Stevenson 1965); analgesic activity was assayed in ICEM:CET (SPF Caw) mice on phenylquinone writhing (Siegmund & Cadmus 1957). Drugs were given orally, suspended in 0·5% Methocel (hydroxypropyl-methyl cellulose 400). Acute toxicity was determined in rats 7 days after oral and intravenous treatment (drugs given as sodium salts). Biological activity was determined and evaluated as previously described (Buttinoni et al 1973).

In-vitro inhibition of prostaglandin synthesis was studied according to Ceserani et al (1979). The concentration used were 0·6, 1·2, 2·4 ng ml^{-1} for racemic indoprofen (n = 11–13) 0·3, 0·6, 1·2 ng ml^{-1} for (*S*)-(+)-enantiomer (n = 11–12) and 24, 48, 96 ng ml^{-1} for (*R*)-(−)-enantiomer (n = 3). The findings were analysed statistically as previously described (Ceserani et al 1979) to obtain KB (KB = dose of indoprofen which reduces the activity of arachidonic acid by 50%, see Furchgott 1972).

Results

Tables 1 and 2 show the anti-inflammatory and analgesic activity and the acute toxicity of racemic indoprofen and its enantiomers. The (*S*)-(+)-isomer is twice as effective and toxic as racemic indoprofen, but the (*R*)-(−)-isomer displayed very little activity and toxicity.

Similar potency ratios were obtained in-vitro. Racemic indoprofen and the (*S*)-(+)-isomer inhibited prostaglandin synthesis with a KB = 2·29 (confidence limits for $P = 0.95$ (1·56–3·37) and 1·27 (0·43–3·74)

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