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An examination of the 'wet dog' shake behaviour in rats produced by acute administration of sodium n-dipropylacetate

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Investigations into the mode of action of the anticonvulsant drug sodium n-dipropylacetate (DPA) have concentrated on its interactions with brain γ-aminobutyric acid (GABA) metabolism (for review see Kupferberg 1980). However, acute and chronic administration of DPA to rodents has been shown to elevate brain concentrations of 5-hydroxyindoleacetic acid (5-HIAA), possibly by stimulating 5-hydroxytryptamine (5-HT) turnover (Hwang & Van Woert 1979). Also, acute administration of DPA in rats causes 'wet dog' shakes (WDS) behaviour (de Boer et al 1977), a behavioural syndrome thought to be an expression of central 5-HT receptor activation (Bedard & Pycock 1977). These observations prompted us to examine the WDS behaviour produced by DPA in more detail, with particular regard to the possible involvement of GABAergic and 5-hydroxytryptaminergic systems.

Male Wistar rats, 150–250 g, were used. For behavioural observations animals were placed individually in an open-topped cage (50 cm × 30 cm × 25 cm) and left undisturbed for 5 min. DPA was then administered and the animal's behaviour observed for the following 30 min. WDS was scored as the number of whole-body shakes occurring during this period. The effects of various drug pretreatments on this behaviour were examined in separate groups of rats. A group of vehicle-pretreated controls was scored with each drug-pretreated group such that, during each experiment, drug-pretreated and control rats were observed alternately. One worker administered drugs and behaviour was scored by a second observer who was unaware of the pre-treatment status of individual rats. All drugs were administered by the intraperitoneal route, dissolved in 0·9% NaCl saline, at a volume of 5 ml kg^{-1}.

Whole-brain GABA was assayed by microdansylation (Briel & Neuhoff 1972) using [^{14}C]GABA as internal standard (Snodgrass & Iversen 1973). 5-HT and 5-HIAA were measured by the method of Curzon & Green (1970). All statistical analysis was by Student's *t*-test.

Drugs: L-Tryptophan, *p*-chlorophenylalanine methyl ester and picrotoxin were obtained from Sigma, sodium n-dipropylacetate from Reckitt & Coleman, morphine sulphate from Macfarlane Smith and chlordiazepoxide from Roche.

Within 3 min of DPA administration (400 mg kg^{-1}) the general activity of the animals increased abruptly. Although some ataxia was apparent at this dose locomotor activity was markedly increased. Additional behavioural changes included episodes of vigorous grooming and the occurrence of whole-body shakes (WDS) which were occasionally of sufficient violence to throw the animal off balance. After 15–20 min, general activity began to subside and at 30 min the animals were sedated, exhibiting ptosis, piloerection and a hunched-back posture. The number of WDS were counted over the 30 min period and the effect of varying the dose of DPA is shown in Fig. 1. The effects of various drug pretreatments on the WDS behaviour elicited by DPA (400 mg kg^{-1}) are shown in Table 1. A lower dose of DPA (300 mg kg^{-1}) was used in the L-tryptophan

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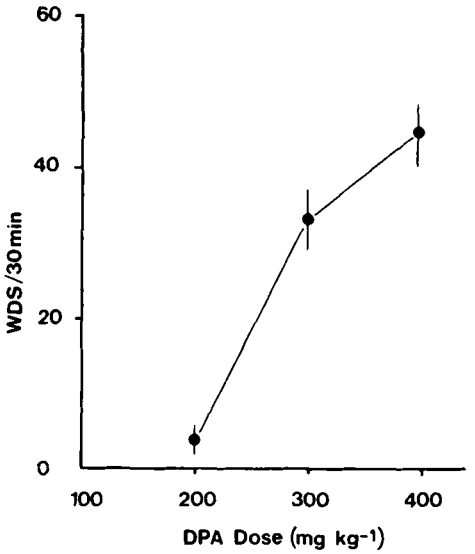

FIG. 1. Dose-dependency of the WDS response. Values are means ± s.e.m. (n = 5) of the number of WDS occurring in the 30 min following DPA administration.

experiment. A subconvulsive dose of picrotoxin reduced the WDS response to 27% of control. A greater blockade (10% of control) was produced by pretreatment with *p*-chlorophenylalanine (pCPA) which was found to reduce whole brain 5-HT levels to 13% of control (n = 3). L-Tryptophan increased the WDS response to 300 mg kg^{-1} DPA (229% of controls). Morphine sulphate decreased the WDS score to 14% of controls and chlordiazepoxide produced a more modest attenuation of the response (48% of controls).

15 min after DPA administration (400 mg kg^{-1}) whole-brain 5-HT levels were unchanged (controls 750 ± 31 ng g^{-1}; DPA 782 ± 36 ng g^{-1}; n = 5) whereas 5-HIAA levels were significantly increased (controls 343 ± 24 ng g^{-1}; DPA 397 ± 11 ng g^{-1}; n = 5, $P < 0.05$). GABA levels were also significantly increased at this time (controls 2·84 ± 0·15 μmol g^{-1}; DPA 3·29 ± 0·08 μmol g^{-1}; n = 4, $P < 0.025$). All values are mean ± s.e.m.

The available evidence suggests that the anticonvulsant properties of DPA are due mainly to a facilitation of GABAergic neurotransmission either by potentiating the postsynaptic response to GABA (Gent & Phillips 1980) or by increasing the amount of GABA available for release from nerve terminals (Iadarola & Gale 1979). The results of our experiments appear to indicate that GABA is also closely involved in the production of WDS behaviour by DPA, since this response was blocked by the GABA antagonist picrotoxin and whole-brain GABA levels were significantly increased 15 min after DPA administration. However, inhibition of 5-HT synthesis by pCPA also blocked the WDS response to DPA and administration of L-tryptophan, which stimulates 5-HT synthesis and increases 5-HT turnover (Eccleston et al 1965), potentiated

Table 1. The effect of various drug pretreatments on the WDS behaviour evoked by DPA. Values are means ± s.e.m. (n = 5) of the number of WDS occurring in the 30 min following a DPA challenge of 400 mg kg^{-1} i.p. (except for the L-tryptophan experiment where 300 mg kg^{-1} DPA was used). Picrotoxin was co-administered with DPA; pCPA was administered on each of the two days immediately preceding DPA challenge; L-tryptophan was administered 30 min before DPA and morphine sulphate and chlordiazepoxide HCl 60 min before DPA. Drug-pretreated groups were compared with their appropriate control groups by Student's *t*-test. All changes were significant ($P < 0.01$).

Drug and dose (mg kg^{-1} i.p.)	WDS score	
	Controls	Drug
Picrotoxin, 1	48 ± 6	13 ± 4
pCPA methyl ester, 400	44 ± 6	8 ± 2
L-Tryptophan, 100	14 ± 2	32 ± 2
Chlordiazepoxide HCl, 5	49 ± 3	20 ± 2
Morphine sulphate, 5	41 ± 6	6 ± 2

the behavioural effects of DPA. The ability of DPA to influence central 5-HT metabolism has been reported by Hwang & Van Woert (1979) who measured increased levels of brain 5-HIAA following DPA administration. In our experiments DPA was found to increase whole-brain 5-HIAA content 15 min after drug administration suggesting that 5-HT turnover had increased.

The effect of a benzodiazepine, chlordiazepoxide, in our experiments appears paradoxical since this class of drug is thought to act by potentiating GABA-ergic transmission (Haefely et al 1975) and might therefore be expected to augment the action of DPA. However, benzodiazepines have been reported to reduce central 5-HT turnover (Saner & Pletscher 1979; File & Vellucci 1978), a property which may explain the attenuation of DPA-induced WDS behaviour by chlordiazepoxide.

Our results indicate an involvement of both GABAergic and 5-hydroxytryptaminergic systems in the control of WDS behaviour evoked by DPA although the complete sequence of events probably includes other types of neuron. For example, we found that morphine also blocked the WDS response, an observation also reported by de Boer et al (1977). These authors also commented on the similarity of the DPA-evoked behaviour to the morphine abstinence syndrome, suggesting the possibility of a complex interaction between GABAergic, 5-hydroxytryptaminergic and opiate systems in the production of WDS behaviour by DPA.

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Blockade by trazodone of naloxone-precipitated jumping in morphine-dependent rats: correlation with brain levels of *m*-chlorophenylpiperazine

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Recent studies have shown that *m*-chlorophenylpiperazine (mCPP) displaces [^{3}H]-5-hydroxytryptamine (5-HT) binding to rat brain membranes (Samanin et al 1980a) and produces pharmacological and biochemical effects indicative of a stimulatory action on postsynaptic 5-HT receptors in the brain (Samanin et al 1979; Garattini 1979).

Since mCPP has been found in rat urine and brain after treatment with trazodone (Melzacka et al 1979; Caccia et al 1981a), a recently introduced antidepressant (Fabre et al 1979), the possibility exists that trazodone exerts 5-HT-like effects through the formation of mCPP. This hypothesis was recently proposed on the basis of pharmacological studies with trazodone in rats (Maj et al 1979, 1980), although these experiments provided no direct evidence that substantial amounts of mCPP were indeed present in the brain of trazodone-treated rats.

In an attempt to obtain more direct information on trazodone's ability to form sufficient mCPP to cause 5-HT-like effects, we measured brain mCPP concentrations in rats treated with doses of trazodone and mCPP producing comparable effects in a test useful to reveal 5 HT-activities. Naloxone-precipitated jumping in morphine-dependent rats was selected since recent findings have shown that this sign is selectively blocked by drugs increasing 5-HT transmission in the brain (Samanin et al 1980b).

Materials and methods

Male CD-COBS rats (Charles River, Italy), about 200 g at the beginning of the experiments, received two intraperitoneal injections of 10 mg kg^{-1} of morphine hydrochloride at 10 a.m. and 6 p.m. on the first day of treatment. The dose of morphine was doubled every other day to reach a total daily dose of 160 mg kg^{-1} on the 7th day. The largest dose was given for 3 more days. On the 11th day the animals received the last injection of morphine at 10 a.m., and 4 h later the abstinence syndrome was precipitated by an intraperitoneal injection of naloxone HCl (1 mg kg^{-1}). Trazodone (12·5 and 25 mg kg^{-1}) and mCPP (0·25, 0·5 and 1 mg kg^{-1}) were administered orally 1 h before the narcotic antagonist. Withdrawal symptoms within 30 min were recorded by observers unaware of the treatments, according to Samanin et al (1980b). The data are expressed as the numbers of positive animals in the various experimental groups, and differences were analysed statistically by the χ^2 test. At the end of the experiments (30 min after naloxone injection), the animals were killed and their brains removed for the determination of trazodone and mCPP by the gas liquid chromatographic method described by Caccia et al (1981b).

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Results and discussion

In agreement with previous findings (Samanin et al 1980b), jumping, together with ptosis and diarrhoea, was mostly observed in the morphine-dependent animals when injected with naloxone. Other signs described in the literature such as wet-dog shakes, flat posture, teeth chattering, salivation, vocalization on touch and dyspnoea were observed, but less frequently. As shown in Table 1, doses of 12·5 and 25 mg kg^{-1} trazodone blocked jumping to the same extent as 0·25 and 0·5 mg kg^{-1} of mCPP. Since comparable brain mCPP concentrations were found after treatment with these doses of trazodone and mCPP, the results suggest that trazodone blocks jumping in morphine-dependent rats through the formation of mCPP.

Table 1. Withdrawal signs and brain mCPP concentrations in trazodone- and mCPP-treated rats. Drugs were given 1 h before naloxone and the animals were killed 30 min after naloxone injection for the determination of trazodone and mCPP brain concentration. See methods for details of morphine dependence induction and assessment. * $P < 0{\cdot}01$ compared with saline (χ^2 test).

Treatment mg kg^{-1} orally	Withdrawal signs jumping	Brain concns (μg g^{-1}) Trazodone	mCPP
0·9% NaCl	16/20	—	—
mCPP 0·25	8/20*	—	0·20 ± 0·06
mCPP 0·5	1/20*	—	0·50 ± 0·07
mCPP 1·0	0/20*	—	1·23 ± 0·25
0·9% NaCl	11/20	—	—
Trazodone 12·5	4/20*	0·44 ± 0·10	0·29 ± 0·06
Trazodone 25·0	2/20*	1·32 ± 0·11	0·65 ± 0·10