Plasma levels of clomipramine and its N-desmethyl metabolite following oral administration of clomipramine in man

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Clomipramine is a tricyclic antidepressant which has been in clinical use for a number of years, however, only limited information is available on blood levels (Faigle & Dieterle, 1973). For this reason, we have determined plasma concentrations of clomipramine together with its *N*-desmethyl metabolite following oral administration of single and multiple doses of clomipramine in man. Analysis of plasma samples was carried out using a modification of the double radio-isotope derivative technique described by Riess (1974) for the quantitative estimation of maprotiline in body fluids.

Five healthy male volunteers received a single oral dose of clomipramine (50 mg) after being fasted overnight. Venous blood samples were collected before and at various times up to 48 h after dosing. Clomipramine appeared rapidly in the plasma and reached peak levels between 2.0 h (37.1 ± 10.4 (s.e. mean) ng/ml) and 4.0 h (38.1 ± 7.8 ng/ml). The mean value of individual peak plasma levels was 44.8 ng/ml (range 22.5–64.6 ng/ml). On the other hand, desmethylclomipramine was not detected in the plasma until 1.0 h after dosing and reached maximum concentrations between 4 and 24 h of 0.5–12.0 ng/ml (mean 5.0 ng/ml). Both parent drug and metabolite were still detectable at 48 h indicating relatively long plasma half-lives for both substances.

In a multi-dose study carried out in nine patients diagnosed clinically as endogenous depressives, clomipramine (25 mg) was administered orally three times daily for four weeks. Venous blood samples were collected immediately prior to the first dose and at 7, 14 and 28 days. Steady-state plasma levels of clomipramine were reached at day 7 and mean individual values ranged from 21.4 to 64.2 ng/ml (mean 38.6 ng/ml). In contrast, mean plasma levels of desmethylclomipramine continued to rise during the treatment period from 48.9 ± 8.4 ng/ml at day 7 to 68.7 ± 17.1 ng/ml at day 28.

Marked individual variation in the ratio between the tertiary amine and its secondary amine metabolite was noted in the patient study indicating the varying abilities of individuals to metabolize clomipramine. In the majority of patients, plasma levels of metabolite were greater than parent drug and since desmethyl-clomipramine possesses biological activity (Sigg, Soffer & Gyermek, 1963; Carlsson, Corrodi, Fuxe & Hökfelt, 1969) it may be a major contributory factor in the overall antidepressant effect of administered clomipramine.

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Inducing dependence by a single administration of morphine in rats

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Studies on acute dependence in rodents have chiefly centred on antagonist-induced jumping in morphine treated mice, by which some workers claim to have assessed dependence following a single injection of opiate (Cheney & Goldstein, 1971; Smits, 1975). However, the validity of such a measure has been disputed (Barthelemy & Jacob, 1972; Kosersky, Harris & Harris, 1974). A conditioned aversion technique, which has been shown to permit reliable assessment of dependence in rats receiving low doses of morphine (Pilcher & Stolerman, 1975) has now been employed to investigate single dose effects.

Rats were adapted to drinking their daily fluid intake in two sessions totalling one hour. The first of

these was for 15 min from 10.00 h and the second was for 45 min from 17.00 hours. After eight days on this schedule, water intakes had stabilized. On day 9 a solution of sodium saccharin (0.1% w/v in distilled water) was presented to all rats in the 15 min session only (medium intake 9.5 ml). Each animal had received a single injection of either morphine hydrochloride or physiological saline at intervals ranging from 1.5 to 24 h before the flavour presentation. All injections were intraperitoneal. Immediately after the 15 min session the rats were injected with either naloxone hydrochloride or saline. Water was provided in the afternoon session and on the following day. On the second day after the first flavour presentation, sodium saccharin was again presented in the 15 min session and water in the afternoon session.

When morphine at 10 mg/kg was given 1.5 h before an injection of naloxone at a dose of either 1.0 or 10 mg/kg, a marked and significant reduction in subsequent saccharin intake was observed (5.9 ml and 6.3 ml respectively; $n=6$, $P<0.03$). With a single dose of morphine at 10 mg/kg, naloxone at 10 mg/kg also suppressed intake (4.5 ml; $n=6$, $P<0.05$) at agonist-antagonist intervals up to 12 hours. When morphine was increased to 30 mg/kg, significant aversions were obtained with naloxone at 0.32; 1.0, 3.2 ($n=6$, $P<0.05$) and 10 mg/kg ($n=12$, $P<0.02$) 24 h later. There was no difference in the degree of aversion at the three lowest doses but the greatest reduction in intake was seen with the highest dose.

Control rats receiving saline before the flavour presentation and naloxone (0.32; 1.0, 3.2 or 10 mg/kg) immediately after it, showed no significant difference in intake in the second flavour trial, when compared with rats receiving saline in both injections. In control rats receiving saline 1.5 h after morphine, a slight but non-significant reduction in intake was seen. However, at intervals of 3 h or longer intake was not suppressed in the morphine-saline groups, even with doses of morphine up to 30 mg/kg.

It appears therefore, that those processes induced by morphine and which are implicated in the aversiveness of withdrawal, are initiated within 1.5 h of first exposure to the opiate in the rat. Moreover, the conditioned aversion technique extends the finding to the rat, that a single low dose of morphine is capable of producing manifestations of dependence.

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Some behavioural and EEG studies on the behavioural depression induced in the rat by ethanolamine O-sulphate, an inhibitor of GABA-transaminase

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Ethanolamine O-sulphate (EOS), an irreversible active-site-directed inhibitor of GABA transaminase (Fowler & John, 1972) when injected intracerebroventricularly in mice elevated whole brain GABA concentrations and induced a behavioural depression characterized by decreased locomotor activity, ptosis and hypothermia (Baxter, Fowler, Miller & Walker, 1973). We have now compared the effects in rats (male, 250 to 350 g) of chlorpromazine (CPZ) injected subcutaneously and EOS, injected intraventricularly (i.v.c.) in three tests known to reveal CPZ-like activity: apomorphine antagonism, shock avoidance conditioning and effects on the electroencephalogram (EEG).

The doses of EOS used in these studies (160 to 320 μg) significantly elevated brain GABA concentrations. Ethanolamine O-sulphate was administered i.v.c. to rats briefly anaesthetized with halothane and the brain GABA concentrations were assayed fluorometrically after 24 h by the methods of Lowe, Robins and Eyerman (1958) and Uchida & O'Brien (1964). GABA concentrations were significantly elevated at all doses ($P\leqslant 0.005$, $n=4$). In a