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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References

BARTHELEMY, C. & JACOB, J. (1972). Naloxone precipitated abstinence after one single injection of morphine and effects of naloxone on a single dose tolerance to morphine. *J. Pharmacol. (Paris)*, **3**, 530.

CHENEY, D.L. & GOLDSTEIN, A. (1971). Tolerance to opioid narcotics: time course and reversibility of physical dependence in mice. *Nature*, **232**, 477–478.

KOSERSKY, D.S., HARRIS, R.A. & HARRIS, L.S. (1974). Naloxone precipitated jumping activity in mice following the acute administration of morphine. *Eur. J. Pharmac.*, **26**, 122–124.

PILCHER, C.W.T. & STOLERMAN, I.P. (1975). Aversive properties of naloxone in morphine treated rats. *Br. J. Pharmac.*, **54**, 235P.

SMITS, S.E. (1975). Quantitation of physical dependence in mice by naloxone-precipitated jumping after a single dose of morphine. *Res. Commun. chem. Pathol. Pharm.*, **10**, 651–661.

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

further test, EOS at 240 μg was found to significantly increase brain GABA concentrations from 2 to 72 h after drug ($P < 0.05$, $n = 3$ to 6).

In the apomorphine test EOS at 240 and 320 μg injected i.v.c. under brief halothane anaesthesia 24 h previously failed to antagonize gnawing induced by apomorphine (5 mg kg^{-1} s.c.) given 30 min prior to observation. In contrast, CPZ given 1 h previously, completely antagonized apomorphine-induced gnawing ($ED_{50} = 2.3$ mg/kg s.c.).

In the shock avoidance conditioning test (shuttle boxes) trained rats received 1 h blocks of conditioning trials (trial interval 1 min) after administration of EOS at 160, 200 or 240 μg ($n = 6$ per treatment) through an indwelling intraventricular cannula. Mean hourly response latencies (RL) were significantly increased ($P \leqslant 0.05$) at 5, 24 and 48 h by 200 and 240 μg. EOS at 160 μg was ineffective. CPZ (2.5 mg/kg s.c.) tested at 1, 3 and 5 h post-drug significantly increased ($P = 0.01$) RL values at all times. No obvious relationship between elevated brain GABA concentrations and behavioural depression was revealed in this test.

EEG studies were undertaken in rats chronically implanted with skull electrodes and an indwelling intraventricular cannula (Goff, Miller, Smith, Smith & Wheatley, 1975). The parietal EEG, recorded on magnetic tape, was analysed (10 s periods at 10 s intervals) by passing it through four broad wave band filters which measured the voltage within each of the following frequencies: 2.4 to 4.0, 4.0 to 7.5, 7.5 to 13.5 and 13.5 to 26.0 Hz (Nos. 1 to 4 respectively). The mean voltage, in hourly blocks was calculated for pre- (2 h) and post-drug periods (CPZ at 0–4 h; EOS at 0–4, 24, 48 h and 7 days). CPZ ($n = 4$) at 8.4 mg/kg s.c., (the ED_{95} in the apomorphine test), significantly increased ($P \leqslant 0.05$) voltages in Filters 1 and 2 and total voltage at all times, but voltages in Filters 3 and 4 were only significantly increased at 1 to 4 h and 2 to 4 h respectively. EOS at 240 μg ($n = 3$) significantly increased ($P < 0.05$) total voltage and voltages in Filters 1 to 3 (at 24 and 48 hours).

The studies have revealed that the behavioural depression associated with increased brain GABA concentrations induced by EOS in rats differs from CPZ-induced depression in some important respects.

References

BAXTER, M.G., FOWLER, L.J., MILLER, A.A. & WALKER, J.M.G. (1973). Some behavioural and anticonvulsant actions in mice of ethanolamine O-sulphate, an inhibitor of 4-aminobutyrate aminotransferase. *Br. J. Pharmac.*, **48**, 681P.

FOWLER, L.J. & JOHN, R.A. (1972). Active-site-directed irreversible inhibition of rat brain 4-aminobutyrate aminotransferase by ethanolamine O-sulphate *in vitro* and *in vivo*. *Biochem. J.*, **130**, 569–573.

GOFF, D., MILLER, A.A., SMITH, R.E., SMITH, S.J. & WHEATLEY, P.L. (1975). Combined EEG recording and intraventricular administration of drugs in the conscious rat. *Br. J. Pharmac.*, **55**, 312P–313P.

LOWE, I.P., ROBINS, E. & EYERMAN, G.S. (1958). The fluorimetric measurement of glutamic decarboxylase and its distribution in brain. *J. Neurochem.*, **3**, 8–18.

UCHIDA, T. & O'BRIEN, R.D. (1964). The effect of hydrazine on rat brain 5-hydroxytryptamine, norepinephrine and gamma-aminobutyric acid. *Biochem. Pharmac.*, **13**, 725–730.

The effects of three tricyclic antidepressants on arterial ^{3}HNA uptake and arterial responsiveness to noradrenaline (NA)

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The tricyclic antidepressant clomipramine has been used in the therapy of obsessional neurosis in which it is demonstrably more effective than either imipramine or amitriptyline (Rack, 1973). Both imipramine and amitryptiline are potent inhibitors of noradrenaline (NA) uptake (Iversen, 1967) and in this study the effects of imipramine, amitriptyline and clomipramine on arterial NA uptake and arterial responsiveness to NA were investigated.

Rat mesenteric arteries were prepared and perfused as previously described (George & Leach, 1973). To measure the uptake of [^{3}H]-NA, the preparation was perfused for 30 min with normal Krebs solution containing (–)-[^{3}H]-NA 0.42 μCi/ml and carrier (–)-NA to give a final NA concentration of 200 ng/ml. Ascorbic acid (20 mg/l) and disodium E.D.T.A. 10 mg/l were added to stabilize (–)-NA. The [^{3}H]-NA content of the arteries was determined as described by Iversen (1963) and radioactivity counted as described by George & Leach (1975). Dose response curves were obtained to NA alone and, to NA in the presence of each of the tricyclic compounds separately. The perfusion concentrations used were 1×10^{-6} M and 1×10^{-8} M. [^{3}H]-NA uptake was measured in the absence of any drug and the effect of each drug on [^{3}H]-NA uptake was determined separately using a range of perfusion concentrations (5×10^{-6} M–1×10^{-8} M). Each of the drugs,