REFERENCES

DIEM, K. & LENTZNER, C. (1970). *Documenta Geigy Scientific Tables*, 7th Edition, p. 583, Basle: J. R. Geigy.
JUDIS, J. (1977). *J. pharm. Sci.*, **66**, 802–806.
MATHER, L. E., LONG, G. J. & THOMAS, J. (1971). *J. Pharm. Pharmac.*, **23**, 359–365.
OLSEN, G. D. (1973). *Clin. Pharmac. Ther.*, **14**, 338–343.
THOMAS, J., LONG, G., MOORE, G. & MORGAN, D. (1976). *Ibid.*, **19**, 426–434.
TUCKER, G. T. (1975). *Int. Anesthesiol. Clin.*, **13**, 33–57.
TUCKER, G. T., BOYES, R. N., BRIDENBAUGH, P. O. & MOORE, D. C. (1970) *Anesthesiology*, **33**, 304–314.
VALLNER, J. J. (1977). *J. pharm. Sci.*, **66**, 447–465.
VALLNER, J. J. & CHEN, L. (1977). *Ibid.*, **66**, 420–421.

'Antagonist'-precipitated withdrawal in the rat after chronic Δ^9-tetrahydrocannabinol treatment

DAVID A. TAYLOR*, MAX R. FENNESSY, *Department of Pharmacology, University of Melbourne, Parkville, Victoria 3052, Australia*

It is well documented that tolerance develops in man (Jones & Benowitz, 1976) and laboratory animals (Paton, 1975) to many of the effects of cannabis extracts or Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive constituent of cannabis. Evidence for dependence on cannabis or Δ^9-THC preparations is less impressive, with most studies reporting lack of abstinence syndromes after chronic treatment (Leite & Carlini, 1974; Snyder, 1971). However, a few investigations have revealed withdrawal signs following the cessation of chronic administration of Δ^9-THC in the monkey (Kaymakcalan, 1972) and man (Jones, 1971; Jones & Benowitz, 1976), and one study has reported naloxone-precipitated withdrawal in rats (Hirschhorn & Rosecrans, 1974). The earlier literature may have discounted a cannabis abstinence syndrome in animals and man because of the inappropriate comparisons made with the more striking phenomena associated with opiate withdrawal. In addition, unlike experience with the opiates, abstinence symptoms to cannabis have not been precipitated because a specific antagonist has not been identified. Previous work has implicated the involvement of serotoninergic mechanisms in several of the actions of Δ^9-THC (Sofia, Dixit & Barry, 1971; Ho & Johnson, 1976; Taylor & Fennessy, 1977). Clomipramine (chlorimipramine), a potent inhibitor of 5-HT uptake (Lidbrink, Jonsson & Fuxe, 1971), appears to antagonize the Δ^9-THC-induced hypothermia and changes in brain monoamines of the rat (Fennessy & Taylor, 1978). We undertook to determine whether rats, chronically treated with Δ^9-THC, exhibit changes in behaviour induced by cessation of treatment or by injections of clomipramine. We observed that administration of clomipramine, but not cessation of Δ^9-THC, produced quantifiable behavioural changes which may indicate the precipitation of a withdrawal response.

All experiments were conducted in a room at an ambient temperature of 21 ± 2° and a 12 h light–dark cycle. Cannulae were implanted into the external jugular veins of individually-caged male Wistar rats, 240–280 g (Fennessy & Taylor, 1977). After recovery for 48 h, rats were divided into two groups of ten, each group receiving intravenous injections of either Δ^9-THC or its vehicle, polyvinylpyrrolidone (PVP, Fenimore & Loy, 1971), twice daily for 10 days. The following schedule of doses of Δ^9-THC and PVP was used:

	Dose (mg kg^{-1})			
	Δ^9-THC		PVP	
Day	a.m.	p.m.	a.m.	p.m.
1	2	2	40	40
2–4	4	4	80	80
5	4	6	80	120
6–10	6	6	120	120

On day 11, each group was further divided into two groups of 5 rats. Rats from all groups were placed individually in 10 litre opaque plastic buckets and allowed to acclimatize for 30 min. One group of the Δ^9-THC-treated and one group of the PVP-treated rats were then injected intraperitoneally with clomipramine HCl, 15 mg kg^{-1}. The other two groups were given injections of normal saline. Overt behaviour, such as jumps, kicks, wet shakes and writhes, were recorded for the next 30 min. The animals were then returned to their home cage and their behaviour observed for the next 13 days. Body temperature was recorded by means of a thermistor probe inserted 6–7 cm into the colon 30 min before and 30 min, 4 and 8 h after the injection of clomipramine or saline, and once daily for the next 13 days. Body weight was measured twice daily throughout the 23 day experi-

* Correspondence.

Table 1. *Effect of clomipramine and saline on the behaviour of rats pretreated with Δ⁹-THC.*

Behavioural signs* (total number observed)	Clomipramine Δ⁹-THC	Clomipramine PVP	Saline Δ⁹-THC	Saline PVP
Writhes	85†	11	0	0
Wet shakes	30†	0	0	0
Jumps	7†	0	0	0
Backward kicks	34†	0	0	0

* The numbers refer to the total number of each behavioural sign in groups of 5 rats observed for the 30 min following clomipramine on the 11th day. Each group of 5 rats had received chronic intravenous injections of Δ⁹-THC or PVP twice daily for the previous 10 days. Clomipramine HCl (15 mg kg^{-1}), dissolved in saline, was injected intraperitoneally.

† $P > 0.05$.

Statistical analysis was performed by calculating the χ^2 value comparing PVP-treated with Δ⁹-THC-treated animals after clomipramnie.

mental period, and 4 and 8 h after the injection of clomipramine or saline on day 11. Food and water were freely available except for the 8 h period of observation on day 11. Food intake was recorded throughout.

Table 1 indicates that rats, chronically treated for 10 days with Δ⁹-THC and challenged with clomipramine exhibit modifications in behaviour which we tentatively suggest may be withdrawal symptoms associated with Δ⁹-THC-induced physical dependence. These responses began 2 min after the injection of clomipramine, reached a peak by 15 min and were waning by 30 min. Quantifiable changes in behaviour consisted of writhing, wet dog shakes, jumping and backward kicking of hind legs. Other responses, difficult to monitor, but apparently characteristic of this withdrawal syndrome, included front paw tremor, fine body tremor, ptosis, chewing movements, excessive grooming, yawning, squealing and ataxia manifested as listing to one side, unsteadiness in gait and sitting up on the posterior for long periods. Clomipramine did not induce this type of behaviour in PVP-treated rats, nor did cessation of Δ⁹-THC treatment in Δ⁹-THC-treated rats. The writhing responses were qualitatively similar to those observed when rats receive intraperitoneal injections of phenylquinone or acetic acid. However, there is a dissimilarity in that the writhes in the Δ⁹-THC-treated animals were more sustained, e.g. one of the rats writhed only three times even though the writhes were maintained for the observation period. The jump and wet shake responses were similar to those seen when rats dependent on morphine are challenged with naloxone (Laska & Fennessy, 1976). The backward kicking movements of the hind legs appear to be a characteristic of this syndrome and occurred in all Δ⁹-THC-treated rats challenged with clomipramine.

The results in Table 2 indicate that, over the period

Table 2. *Percentage changes in body weight, food intake and body temperature of rats chronically treated with Δ⁹-THC before and after 'withdrawal'.*

No. of days of treatment	Clomipramine Δ⁹-THC	Clomipramine PVP	Saline Δ⁹-THC	Saline PVP
	Body weight: % of day 1			
1	100	100	100	100
11	97·66 ±1·85*	112·16 ±3·43	102·40 ±1·98*	108·36 ±1·71
12	98·35 ±1·94*	111·48 ±2·93	104·23 ±1·93	108·83 ±1·74
23	110·92 ±2·18	121·60 ±4·64	117·82 ±2·70	118·51 ±3·35
	Food intake: % of combined PVP—days 1–10.			
1–10	80·84 ±5·00*	102·34 ±6·61	79·91 ±4·69*	98·13 ±5·27
11–23	90·19 ±4·70	99·53 ±7·75	90·65 ±3·68	95·33 ±4·22
	Body temperature: % of day 1			
1	100	100	100	100
10	101·29 ±0·38	101·64 ±0·43	100·97 ±0·36	100·75 ±0·55
11	100·36 ±0·35	100·16 ±0·48	100·67 ±0·42	100·65 ±0·50
12	100·16 ±0·45	100·03 ±0·55	99·95 ±0·42	99·70 ±0·53
23	99·73 ±0·25	100·13 ±0·42	99·68 ±0·36	99·73 ±0·54

All values are expressed as percentages ± relative s.e. of control values. The control food intake is the amount of food consumed by PVP-treated rats over the first 10 days. This was 21·4 ± 0·7 g day^{-1} per rat. The body weights and temperatures are expressed as percentages of the mean values of each group on day 1. The mean body weight and temperature of all groups on day 1 were 257·7 ± 6·1 g and 37·22 ± 0·06°, respectively.

* $P < 0.05$ compared with respective PVP-treated controls.

of chronic administration, Δ⁹-THC reduced both food intake and body weight but had minimal effect on body temperature. Body weight and food intake in Δ⁹-THC-treated rats were always significantly less ($P < 0.05$) than those in PVP-treated rats until cessation of the Δ⁹-THC treatment. Both measurements then increased up to day 23 when no differences were noted between any of the groups. During the 'withdrawal' induced by clomipramine on the 11th day, body weight and food intake were not affected and diarrhoea did not occur.

These results demonstrate that characteristic changes in behaviour occur when clomipramine is injected into rats chronically treated with increasing doses of Δ⁹-THC over 10 days. Similar responses were not observed in control PVP-treated rats or on cessation of Δ⁹-THC treatment. The dose of 15 mg kg^{-1} of

clomipramine has been shown to block 5-HT uptake preferentially in central serotoninergic neurons (Lidbrink & others, 1971) and to antagonize the Δ^9-THC-induced hypothermia and changes in brain monoamines of the rat (Fennessy & Taylor, 1978). Since a blocker of 5-HT uptake induces these overt changes in behaviour in rats chronically treated with Δ^9-THC, and because Δ^9-THC has been shown to modify central serotoninergic mechanisms (Sofia & others, 1971; Ho & Johnson, 1976; Fennessy & Taylor, 1977; Taylor & Fennessy, 1977), we suggest that clomipramine interferes with the modified dynamics of the serotoninergic system established after chronic Δ^9-THC treatment. This interference would be expected to increase suddenly the concentration of 5-HT in the vicinity of neurons and, if 5-HT release has been decreased during the Δ^9-THC treatment, it would be anticipated that postsynaptic receptors exhibit supersensitivity. This effect, possibly a type of rebound hyperexcitability (Collier, 1968), is manifested as the 'withdrawal' syndrome consisting of writhing, jumping, kicking and wet shakes.

Effects on body weight and food intake induced by chronic Δ^9-THC were not affected by precipitated 'withdrawal' induced by clomipramine. However, since weight loss and hypothermia are measures of morphine dependence (Hosoya, 1975), these effects may indicate a dissimilarity between the withdrawal from opiates and that from Δ^9-THC.

In conclusion, we suggest that a state of physical dependence may be induced in rats by chronic administration of Δ^9-THC. The mechanism of this syndrome may be associated with interference of serotoninergic mechanisms, since a 'withdrawal' syndrome is precipitated by clomipramine, a blocker of 5-HT uptake. On the other hand, a 'withdrawal' response is not observed on cessation of chronic Δ^9-THC treatment. This absence of effect may be due to the high lipid solubility and consequent long biological half-life of Δ^9-THC (Kruez & Axelrod, 1973). Even though clomipramine does precipitate a 'withdrawal' response in this study, we are reluctant to classify it as a specific antagonist of Δ^9-THC in a similar manner as naloxone is an antagonist of the opiate receptor.

We thank the National Health and Medical Research Council of Australia for support, Dr Monique Braude for the Δ^9-THC and Ciba-Geigy Australia for the clomipramine.

March 22, 1978

REFERENCES

COLLIER, H. O. J. (1968). *Nature*, **220**, 228–231.

FENIMORE, D. C. & LOY, P. R. (1971). *J. Pharm. Pharmac.*, **23**, 310.

FENNESSY, M. R. & TAYLOR, D. A. (1977). *Br. J. Pharmac.*, **60**, 65–71.

FENNESSY, M. R. & TAYLOR, D. A. (1978). *Ibid.*, **60**, 267–274.

HIRSCHHORN, I. D. & ROSECRANS, J. A. (1974). *Psychopharmac.*, **36**, 243–253.

HO, B. T. & JOHNSON, K. M. (1976). In: *Marihuana: Chemistry, Biochemistry and Cellular Effects*, pp. 367–382. Editor: Nahas, G. G. New York–Heidelberg–Berlin: Springer-Verlag.

HOSOYA, E. (1975). In: *Methods in Narcotic Research*, pp. 261–291. Editors: Ehrenpreis, S. & Neidle, A., New York; Dekker.

JONES, R. T. (1971). *Ann. N.Y. Acad. Sci.*, **191**, 155–165.

JONES, R. T. & BENOWITZ, N. (1976). In: *The Pharmacology of Marihuana*, pp. 627–642. Editors: Braude, M. C. & Szara, S. New York: Raven.

KAYMAKCALAN, S. (1972). In: *Cannabis and Its Derivatives*, pp. 142–147. Editors: Paton, W. D. M. & Crown, J. London: Oxford University Press.

KRUEZ, D. S. & AXELROD, J. ((1973). *Science*, **179**, 391–393.

LASKA, F. J. & FENNESSY, M. R. (1976). *Clin. exp. Pharmac. Physiol.*, **3**, 587–598.

LEITE, J. R. & CARLINI, E. A. (1974). *Psychopharmac.*, **36**, 133–145.

LIDBRINK, P., JONSSON, G. & FUXE, K. (1971). *Neuropharmac.*, **10**, 521–536.

PATON, W. D. M. (1975). *A. Rev. Pharmac.*, **15**, 191–220.

SOFIA, R. D., DIXIT, B. N. & BARRY, H. (1971). *Life Sci.*, **10**, 425–436.

SNYDER, S. H. (1971). *Uses of Marihuana*, New York: Oxford University Press.

TAYLOR, D. A. & FENNESSY, M. R. (1977). *Eur. J. Pharmac.*, **46**, 93–99.