Effect of Δ^9-THC on Ethanol Withdrawal in Mice

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Summary. Δ^9-THC (10 mg/kg, i.p.) administered to mice immediately after withdrawal from a 3-day exposure to ethanol vapor was found to intensify withdrawal reactions. No effect was seen when Δ^9-THC was administered chronically during the exposure to ethanol.

The abuse of alcohol in many cultures remains a monumental problem socially and physiologically. The treatment of postalcoholic symptoms is presently the most promizing approach to the problem, though most drugs used for this purpose are either partially effective or have side-effects of themselves. Several studies[2-4] investigating the combined effects of ethanol and marijuana or its active constituent (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC) have presented evidence that a cross-tolerance exists between these two drugs of abuse. In one study[3] it was found that synhexal, a synthetic derivative of Δ^9-THC, was beneficial in treating withdrawal symptoms from the use of alcohol. Both compounds elicit depressant effects but only alcohol is known to produce physical dependence, withdrawal from which is accompanied by anxiety, tremulousness and nausea.

A method[5] recently developed to quantitate accurately the alcohol withdrawal reaction in mice has been useful in the evaluation of the effects of drugs, known to alter neurotransmission, on withdrawal from ethanol[6]. The following study was undertaken to determine whether Δ^9-THC, by modifying physical dependence on ethanol, could effect the withdrawal response or could modify the withdrawal reaction without necessarily effecting the addiction process; antagonism of the response would be useful in the treatment of the alcohol withdrawal syndrome.

Materials and methods. Male Yale-Swiss mice of average weight 25–30 g were exposed to a constant level of alcohol vapor using a chamber similar in design to that reported by GOLDSTEIN[5] but with the following modifications: A fan was not installed in the chamber since sufficient circulation was achieved by the air flow (20 CFH) from a continuous duty pressure pump (Neptune Dyna-Pump, Model No. 4K, Neptune Products, Inc., Dover, N. J.); ethanol was delivered into the flask at a rate of 76.3 mg/min by a Sage Syringe Pump, Model 355 (Orion Research, Cambridge, Ma.) which resulted in a vapor concentration of 10.5–11.5 mg/l air within the chamber.

The mice (in groups of 24) were placed in 2 colony boxes containing bedding, within the chamber; they were exposed to the vapor continuously for 3 days except for 1 to 2 h each morning at which time they were given food, water and daily injections (see Table I for injection schedule); samples were also taken to determine blood ethanol levels. A priming dose of ethanol in saline (1.25 g/kg) was given i.p. only on the first day before placing the animals in the chamber; pyrazole (68 mg/kg) in saline was administered daily except on the day of withdrawal. Δ^9-THC was suspended in 4% Tween-80 in saline and 10 mg/kg was administered orally using an 18 ga intubation needle. In the acute study, Δ^9-THC was given immediately upon withdrawal to half of the mice; the remaining animals (vehicle control) received an equivalent volume of 4% Tween-80 in saline. In the chronic study,

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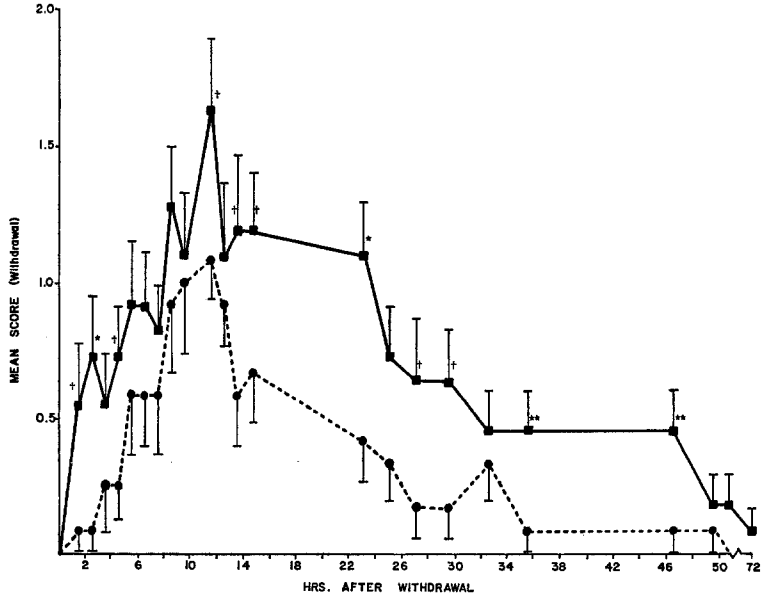

Fig. 1. Effects of acute administration of Δ^9-THC on withdrawal from ethanol. 24 mice were exposed to ethanol for 3 days; of the 23 mice surviving, 11 received 10 mg/kg of Δ^9-THC (solid line) and 12 received the vehicle (dotted line) immediately after withdrawal (see Methods). The points represent the mean scores and the vertical bars are the SEM. * $P < 0.02$; ** $p < 0.05$; + $p < 0.1$ (Student t-test).

Table I. Injection schedule for mice exposed to ethanol vapor

Time[a] (day)	Acute study		Chronic study	
	Control	Δ^9-THC	Control	Δ^9-THC
1	Ethanol, pyrazole	Ethanol, pyrazole	Ethanol, pyrazole, vehicle[b]	Ethanol, pyrazole, Δ^9-THC in vehicle[b]
2	Pyrazole	Pyrazole	Pyrazole, vehicle[b]	Pyrazole, Δ^9-THC in vehicle[b]
3	Pyrazole	Pyrazole	Pyrazole, vehicle[b]	Pyrazole, Δ^9-THC in vehicle[b]
4 (after withdrawal)	Vehicle[b]	Δ^9-THC in vehicle[b]	—	—

[a] The animals were exposed to ethanol vapor continuously for 3 days. [b] Vehicle: 4% Tween-80 in saline.

Δ^9-THC was given daily with the pyrazole injections except on the day of withdrawal, while the controls received pyrazole together with 4% Tween-80 in saline.

The withdrawal reaction was graded hourly for 15 to 16 h, after exposure to alcohol, using the previously described method of convulsions upon handling[5]. The convulsions were elicited by holding the mouse by the tail and when necessary, rotating the mouse to evoke a response. They were then assigned a score, depending upon the intensity of their reaction, as follows: 1 = tonic convulsion when the mouse is lifted and given a gentle 180° turn; 2 = tonic-clonic convulsion elicited by a gentle spin, or tonic convulsion when lifted without turning; 3 = tonic-clonic convulsion not requiring any spin; 4 = violent tonic-clonic convulsion, often continuing after release of the mouse. The mean score and standard error were determined for each interval and plotted against time.

Ethanol levels were determined as previously described[7] using a Varian 2100 gas chromatograph with a dual channel flame ionization detector, and a 6 ft, $^1/_4$ inch. I.D. U-shaped glass column using nitrogen as the carrier gas at a rate of 18 ml/min. The chamber alcohol level was determined daily by assaying 1-ml aliquots of the vapor withdrawn from the sampling port; standards, containing a known concentration of ethanol vapor, were analyzed simultaneously. The blood ethanol levels of the mice were determined indirectly by injecting an air pocket at the nape of the neck, then sampling as above. The partition coeficient of ethanol between the blood to the air pocket is 1850:1.

To determine if Δ^9-THC alone or in combination with pyrazole could cause seizures in animals not exposed to ethanol, two additional studies were made. 12 mice were pretreated for 3 days with pyrazole as described above; 24 h after the last injection, Δ^9-THC (10 mg/kg) was administered (see above) and the mice were immediately scored for convulsions on handling. In another experiment, Δ^9-THC was administered to mice not pretreated with pyrazole, then scored.

Results and discussion. With the use of pyrazole, the ethanol blood level during the period of exposure remained stable at 1.47 mg/ml $\pm$ 0.18 (SEM). This level of ethanol was sufficient for intoxication yet low enough to minimize the mortality rate. The animals were deprived of food and water while being exposed to the vapor to eliminate metabolic fluctuations between the mice; this resulted in an approximate 20% weight loss which was soon recovered after withdrawal.

Figure 1 shows the effects of an acute oral dose (10 mg/kg) of Δ^9-THC (given immediately after withdrawal from ethanol) on the withdrawal reaction; there was a definite enhancement of the severity of the seizures as compared

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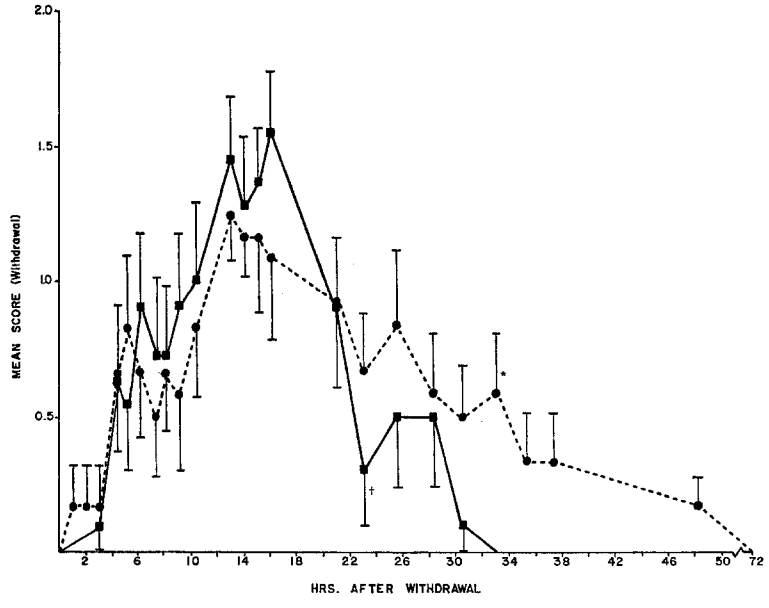

Fig. 2. Effects of chronic administration of Δ^9-THC on withdrawal from ethanol. 24 mice were exposed to ethanol for 3 days; one-half received 10 mg/kg of Δ^9-THC daily (solid line) and the other half received the vehicle (dotted line) (see Methods). Of the Δ^9-THC group, 11 survived the exposure period and 10 recovered from withdrawal; + indicates one death during withdrawal. The points represent the mean scores and the vertical vars are the SEM. * $P < 0.05$ (Student t-test).

Table II. Ethanol levels in blood following withdrawal from exposure to ethanol vapor and acute or chronic treatment with Δ^9-THC (see Methods)

Time after withdrawal (h)	Blood ethanol (mg/ml)			
	Acute		Chronic	
	Vehicle	Δ^9-THC	Vehicle	Δ^9-THC
1.5	0.86 ± 0.04	0.85 ± 0.08	1.08 ± 0.27	1.09 ± 0.16
2.0	0.75 ± 0.04	0.74 ± 0.06	0.96 ± 0.26	0.96 ± 0.16
3.0	0.53 ± 0.05	0.54 ± 0.03	0.75 ± 0.24	0.70 ± 0.16
4.0	0.35 ± 0.06	0.39 ± 0.04	0.57 ± 0.22	0.46 ± 0.13
5.0	0.23 ± 0.06	0.24 ± 0.04	0.44 ± 0.23	0.31 ± 0.10

Each value represents the mean ± SEM of 3 animals. All values obtained with Δ^9-THC are not significantly different from the corresponding control (Student t-test).

to the control animals receiving the vehicle (acute study, Day 4, Table I); the period for recovery was also prolonged in the Δ^9-THC group. To determine whether or not this effect was due to properties intrinsic to Δ^9-THC (as seen with several other compounds[6]), convulsions on handling were scored in separate groups of animals not exposed to ethanol but were given Δ^9-THC with or without 3-day pretreatment with pyrazole. In either case the scores were minimal (0.6 to 0.7) and began to diminish 1 to 2 h after administration; these findings could not, therefore, account for the prolonged facilitation of withdrawal seizures by Δ^9-THC seen in Figure 1. Our results contrast with those reported previously[3] in which synhexyl, a synthetic THC derivative, lessens withdrawal symptoms in alcoholics. On the other hand, the above data indirectly confirm the findings of GOLDSTEIN[6] indicating that drugs which modify brain catecholamine levels intensify ethanol withdrawal seizures. Δ^9-THC has been shown to manifest a dose-dependent modification of brain norepinephrine levels in mice[8,9]. Moreover, a fluctuation of norepinephrine and dopamine levels in the brain during the first 12 h of withdrawal was observed in mice exposed to ethanol vapor for 3 days[10]. Recently, it was found that Δ^9-THC also facilitates minimal seizures induced by pentylenetetrazol (PTZ)[11]. Reserpine which is known to enhance PTZ convulsions[12] has also been reported to facilitate alcohol withdrawal seizures[6].

When Δ^9-THC was given chronically to mice undergoing ethanol addiction (chronic study, Days 1 to 3, Table I), the resulting withdrawal response was not significantly different from that seen in the vehicle control (Figure 2).

To examine the possibility that Δ^9-THC could effect the disappearance of ethanol after withdrawal, blood ethanol levels were monitored during this period. As indicated in Table II, no significant difference was found between the vehicle control and the THC group in either the acute or chronic study.

Our findings indicate variable interactions between Δ^9-THC and ethanol: acute injection of Δ^9-THC affects the withdrawal, whereas chronic administration produces no significant effect on the dependence and hence the withdrawal. This implies that addiction and withdrawal are manifestations of different biological processes. It is hoped that these studies will stimulate further investigation into the combined effects of alcohol and marihuana.

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Sur l'inversion de l'action hypertensive de la prostaglandine $F_{2\alpha}$, chez le rat[1]

Reversal of the Pressor Effect of $PGF_{2\alpha}$ in the Rat

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Summary. In normal rats, prostaglandin $F_{2\alpha}$ is a potent vasoconstrictor. The corresponding systemic hypertension is progressively attenuated by tachyphylaxis, revealing a longlasting fall of blood pressure. Tachyphylaxis is accelerated by previous injection of arachidonic acid and bradykinin. The vasodepressive activity of $PGF_{2\alpha}$ is inhibited by indomethacin.

Les prostaglandines E et A sont vasodilatatrices et hypotensives. Par contre, l'activité cardiovasculaire de la prostaglandine $F_{2\alpha}$ ($PGF_{2\alpha}$) est variable selon l'espèce animale: la $PGF_{2\alpha}$ est vasodépressive chez le Chat et le Lapin, hypertensive chez le Chien[2]. Chez le Poulet anesthésié, elle déclenche selon l'animal et la dose, des modifications de trois types: hypertension artérielle, hypotension ou réponse biphasique hypertension-hypotension. Chez le Poulet spinal, elle est uniquement hypertensive[3]. Chez le Rat, elle augmente la pression artérielle suite à son pouvoir vasoconstricteur périphérique[2,4–6].

En étudiant chez le Rat les interactions entre les activités cardiovasculaires de la bradykinine et celles de la $PGF_{2\alpha}$, nous avons observé que trois conditions expérimentales au moins permettent d'inverser l'hypertension artérielle liée à cette prostaglandine, la transformant en une action hypotensive. En voici la description.

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