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Cross-tolerance development to the prolongation of pentobarbitone-induced sleep by Δ^8-tetrahydrocannabinol and 11-hydroxy-Δ^8-tetrahydrocannabinol in mice

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Repeated administration (5 mg kg^{-1} day^{-1} i.v.) of Δ^8-tetrahydrocannabinol and its active metabolite, 11-hydroxy-Δ^8-tetrahydrocannabinol caused tolerance to develop to their prolonging effect on pentobarbitone-induced sleep in mice. Reciprocal cross-tolerance also developed after seven daily doses of these cannabinoids. The magnitude of the tolerance developed by the metabolite was greater than that by Δ^8-tetrahydrocannabinol. The results suggest that 11-hydroxy-Δ^8-tetrahydrocannabinol plays an important role both in the sleep-prolonging effect of Δ^8-tetrahydrocannabinol and its tolerance development.

Tetrahydrocannabinol (THC) is known to potentiate barbiturate-induced sleep. This is considered to be a direct action of the cannabinoid on the central nervous system and not the inhibition of barbiturate metabolism in the liver (Kubena & Barry 1970; Watanabe et al 1981).

Development of tolerance to the pharmacological effects of THC is well recognized in animals (Wikler 1976). However, the mechanism of tolerance development to THC's prolonging effect on barbiturate-induced sleep has not been fully elucidated. Rating et al (1972) reported that subchronic administration of Δ^8-THC led to the attenuation of the prolonging effect of the cannabinoid on hexobarbitone-induced sleeping time in rats. Sofia & Barry (1973) also reported attenuation of the hexobarbitone-induced sleep prolonging effect of Δ^8-THC by its repeated administration to mice. Many investigators have reported on the metabolic activation of Δ^9-THC (Christensen et al 1971; Lemberger et al 1972; Gill et al 1973; Wilson & May 1975). However, limited information is available concerning the role of metabolites in the tolerance development of THC. Recently we reported that repeated administrations of Δ^8-THC and its major and active metabolite, 11-hydroxy-Δ^8-THC (11-OH-Δ^8-THC) led to tolerance development to the pentobarbitone-induced sleep-prolonging effect in mice (Watanabe et al 1982). With respect to the role of metabolites in tolerance development to THC, cross-tolerance development to the cataleptogenic and hypothermic effects between Δ^8-THC and 11-OH-Δ^8-THC has been reported (Watanabe et al 1983; Yamamoto et al 1985) and the magnitude of tolerance development produced by the metabolite was greater than that produced by the parent compound, Δ^8-THC. We have investigated the development of tolerance to the phenobarbitone-induced sleep-prolonging effect of Δ^8-THC and of 11-OH-Δ^8-THC.

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Materials and methods

Animals and drugs. Male ddN mice, 20 to 30 g were used. Δ^8-THC and 11-OH-Δ^8-THC were prepared as described by Watanabe et al (1980). Sodium pentobarbitone was purchased from Tokyo Chem. Ind. (Tokyo, Japan). Cannabinoids were suspended in 0·9% NaCl (saline) containing 1% polysorbate (Tween) 80 and injected intravenously (i.v.) through the tail vein of mice. Sodium pentobarbitone was dissolved in saline and injected intraperitoneally (i.p.) at a dose of 50 mg kg^{-1}. The experiments were made at an ambient temperature of 22–24 °C.

Drug treatments. Each animal in two groups of 60 mice received daily a 5 mg kg^{-1} i.v. dose of Δ^8-THC or 11-OH-Δ^8-THC while those in a group of 90 mice received the vehicle (1% Tween 80–saline). On the 2nd, 4th and 8th days, 30 mice (vehicle-treated) were withdrawn and divided into three groups of 10 mice each. Each group was injected with the vehicle or 5 mg kg^{-1} i.v. of Δ^8-THC or 11-OH-Δ^8-THC. On the same days, 20 mice (cannabinoid-pretreated) were withdrawn and divided into two groups of 10 mice each. Each group was injected with 5 mg kg^{-1} i.v. of Δ^8-THC or 11-OH-Δ^8-THC. Pentobarbitone was injected 20 min after the last injection of the vehicle or the cannabinoids. Duration of the loss of the righting reflex was used as an index of sleep.

Statistics. The statistical significance of difference was calculated using Student's *t*-test.

Results and discussion

The development of cross-tolerance to the pentobarbitone-induced sleep-prolonging effect of Δ^8-THC and 11-OH-Δ^8-THC is shown in Fig. 1. On the 2nd day, neither tolerance nor cross-tolerance was observed with either Δ^8-THC or 11-OH-Δ^8-THC, a result that differs from the hypothermic effect of the cannabinoids since acute tolerance developed to the hypothermic effect of these cannabinoids under the same conditions (Yamamoto et al 1985). On the 4th day, no significant attenuation in the sleep-prolonging effect of 11-OH-Δ^8-

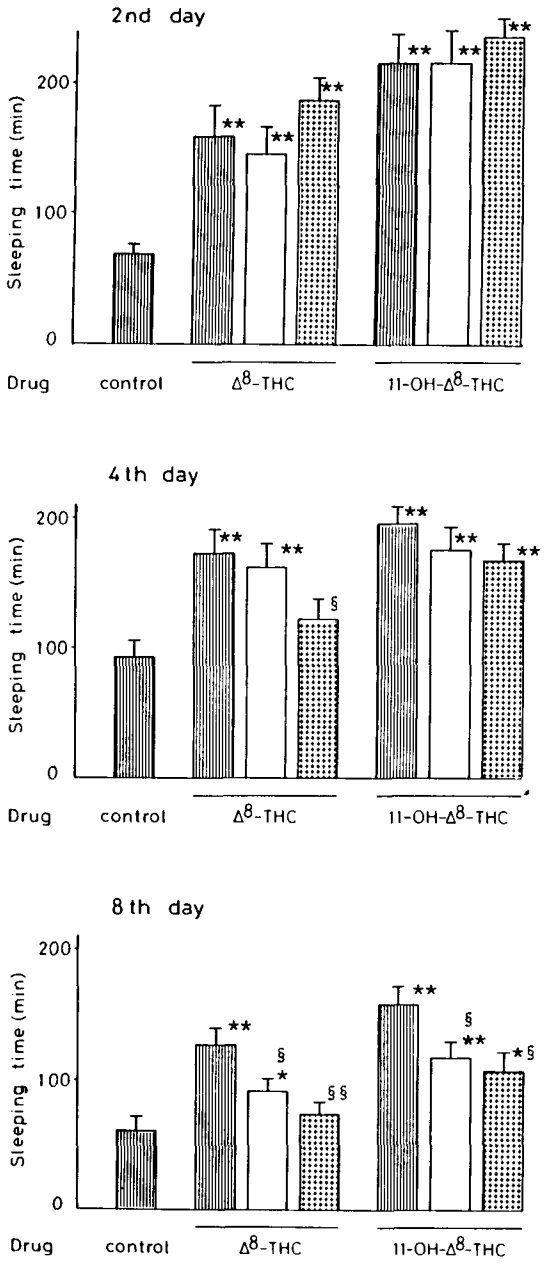

FIG. 1. Cross-tolerance experiment in the pentobarbitone-induced sleep-prolonging effect of Δ^8-THC and 11-OH-Δ^8-THC. Open columns; Δ^8-THC-pretreated, hatched columns; vehicle-pretreated and stippled columns; 11-OH-Δ^8-THC-pretreated mice. Δ^8-THC and 11-OH-Δ^8-THC were administered 5 mg kg^{-1} day^{-1} i.v. for 8 days. Pentobarbitone (50 mg kg^{-1} i.p.) was administered 20 min after the last injection of the vehicle or the cannabinoids. * and ** indicate level of significance as $P < 0.05$ and <0.01, respectively, compared with control group. § and §§ indicate level of significance as $P < 0.05$ and <0.01, respectively, compared with corresponding vehicle-pretreated group.

THC was observed in both groups of cannabinoid-pretreated mice. On the same day, the sleep-prolonging effect of Δ^8-THC was significantly attenuated in 11-OH-Δ^8-THC-pretreated mice. The mean sleeping time (122 ± 15 min) in the 11-OH-Δ^8-THC-pretreated group was significantly shorter than that (173 ± 17 min) in the vehicle-pretreated group, and not significantly different from the control group (93 ± 13 min). The results indicated that 11-OH-Δ^8-THC-pretreated mice were cross-tolerant to Δ^8-THC, but Δ^8-THC-pretreated mice were not cross-tolerant to 11-OH-Δ^8-THC on the 4th day. On the 8th day, the sleep-prolonging effects of Δ^8-THC (92 ± 9 min) and 11-OH-Δ^8-THC (109 ± 15 min) were significantly attenuated compared with the corresponding vehicle-pretreated groups (Δ^8-THC, 127 ± 12 min; 11-OH-Δ^8-THC, 158 ± 13 min), indicating that tolerance developed to the sleep-prolonging effects of these cannabinoids. Reciprocal cross-tolerance also developed to the sleep-prolonging effects of Δ^8-THC and 11-OH-Δ^8-THC on the 8th day. Δ^8-THC (5 mg kg^{-1} i.v.) did not show any prolongation of pentobarbitone-induced sleeping time in 11-OH-Δ^8-THC-pretreated mice. The sleep-prolonging effect of 11-OH-Δ^8-THC (5 mg kg^{-1} i.v.) was significantly attenuated in Δ^8-THC-pretreated mice (118 ± 12 min) compared with that in vehicle-pretreated mice (158 ± 13 min), although the sleeping time was significantly longer than that (61 ± 10 min) in the control group.

The precise mechanism of the tolerance development to the sleep-prolonging effect of Δ^8-THC and of 11-OH-Δ^8-THC is not clear at present. Hypothermia is known to reduce drug metabolism (Vessell 1968) and to prolong barbiturate-induced sleeping time (Raventos 1938). Development of tolerance to the hypothermic effect of Δ^8-THC and 11-OH-Δ^8-THC after daily administration (5 mg kg^{-1} day^{-1} i.v.) has been reported (Watanabe et al 1982). Therefore, development of tolerance to the hypothermic effect of these cannabinoids may also lead to the attenuation of the prolongation of barbiturate-induced sleep by the cannabinoids. Change in the hepatic microsomal drug metabolizing enzymes is also known to affect barbiturate-induced sleeping time. Daily administrations of Δ^8-THC and 11-OH-Δ^8-THC lead to the enhancement of the hepatic microsomal drug metabolizing enzymes (Watanabe et al 1986). Enhancement of drug metabolism may also be a part of the mechanism.

The present study has shown that cross-tolerance between Δ^8-THC and 11-OH-Δ^8-THC develops to their pentobarbitone-induced sleep-prolonging effect in mice, and that the magnitude of the tolerance development produced by the metabolite was greater than that by Δ^8-THC. The results suggest that 11-OH-Δ^8-THC is involved in both the sleep-prolonging effect and the tolerance development of Δ^8-THC.

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Effects of three new anthracyclines and doxorubicin on the rat isolated heart

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The acute cardiac toxicity of three second-generation anthracycline analogues and doxorubicin was compared in a model of the rat isolated Langendorff perfused heart. The drugs, doxorubicin (DX), 4-epi-doxorubicin (4'EDX), 4-demethoxy-daunorubicin (4DMDR) and 4'-deoxy-doxorubicin (4'dxDX) were infused for 40 min at a concentration of 26 μM into the isolated hearts. All four compounds significantly reduced cardiac work and its first derivative. The time to 50% decrease in work (TW50) was respectively 36, 23, 9 and 7 min for DX, 4'EDX, 4'dxDx and 4DMDR. The three anthracycline derivatives, but not DX, significantly increased coronary resistance. Heart rate was reduced by all compounds compared with baseline, but not compared with controls. Rhythm disturbances were seen with all five hearts perfused with 4DMDR, which stopped beating before 40 min; 2/5 hearts in the 4'EDX group and 1/5 hearts in the 4'dxDX group also stopped before the end of perfusion. All the compounds reached concentrations in the myocardium 8 to 50 times higher than in the perfusing medium. The more cardiotoxic the compound, the higher was its myocardial concentration; a significant correlation was found for all four agents. Noradrenaline was never measurable in the perfusate of control and DX hearts; perfusion with the three anthracycline derivatives caused some release, but the pattern was not clearcut and the maximum concentrations attained in the perfusate were relatively low ($\leq 1{\cdot}6 \times 10^{-9}$ M). In conclusion, in the rat isolated perfused heart, the early cardiotoxicity induced by equimolar concentrations of the three anthracycline compounds was greater than that induced by DX and was directly related to drug accumulation in the myocardium. Catecholamines do not seem to have a major role in the development of toxicity in this model.

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The characteristics of anthracycline-induced cardiac damage, which often limit the use of such drugs in patients, were reviewed by Lenaz & Page (1976). The cardiotoxicity of the anthracyclines is best divided into acute and chronic (Bristow et al 1978a; Doroshow et al 1979). The acute cardiac toxicity of anthracyclines in man can appear within hours or days after single or multiple treatment and includes a pericarditis-myocarditis syndrome, left ventricular dysfunction and arrhythmias; chronic toxicity is a severe cardiomyopathy dependent on the cumulative dose. Acute toxic effects of doxorubicin (DX), such as increased coronary resistance, ventricular arrhythmias and a decrease in cardiac performance, have been observed in several animal species, in-vitro and in-vivo, and in patients (Bristow et al 1978b). As these cardiac lesions have some similarities, a common mechanism was proposed for the acute and chronic toxicity of DX, in which the release of vasoactive substances, like histamine and catecholamines, is believed to play a role (Bristow et al 1980, 1981).