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Possible mediation of catecholaminergic pathways in the antinociceptive effect of an extract of *Cannabis sativa L.*

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Abstract. An extract of cannabis (5 and 15 mg/kg expressed as Δ^9-THC) orally administered to rats caused an elevation of the nociceptive threshold (tail-flick latency and vocalization tests). Naloxone and naltrexone (blockers of μ-type opiate receptors) as well as MR 1452 (blocker of κ opiate receptors) did not prevent the antinociceptive effect of cannabis when used at the dose of 2 mg/kg SC; only a high dose (10 mg/kg SC) of these narcotic antagonists partially blocked cannabis antinociception. ICI 154, 129, an antagonist of δ-type opiate receptors, failed to prevent the cannabis-induced rise in nociceptive threshold when used at a dose of 2 mg/kg SC but produced a significant effect at 10 mg/kg SC. While the role of opiate receptors does not seem fundamental to cannabis antinociception, the clear-cut effectiveness shown by 6-hydroxydopamine (a neurotoxin which causes a degeneration of catecholamine-containing terminals) in reducing cannabis antinociception is indicative of a participation of catecholamines in the phenomenon.

Key words: Cannabis – Catecholamines – Antinociception – 6-Hydroxydopamine – Narcotic antagonists

The literature offers a variety of reports concerning the antinociceptive effect elicited in laboratory animals by cannabis and its active constituents (Buxbaum 1972; Sofia and Barry 1972; Chesher et al. 1973; Sofia et al. 1973; Sofia et al. 1975; Wilson and May 1975; Bloom et al. 1977; Bloom and Dewey 1978; Tulunay et al. 1981). Little is known, however, about their mechanism (s) of action and about the interaction with neurotransmitters involved in nociceptive transmission.

In previous work (Ferri et al. 1981) we reported the involvement of catecholamines, particularly dopamine, in some behavioral effects of an extract of *Cannabis sativa L.* in the rat (rhythmic head movements, intermittent gnawing and sniffing, cataleptic state alternating with atonic muscular prostration). This and an earlier report (Ferri et al. 1977) again showing catecholaminergic mediation in antinociception elicited in the rabbit by another hallucinogenic drug, mescaline, prompted us to study the effects of pharmacological manipulation of catecholaminergic pathways on the antinociceptive activity of the cannabis extract. Additionally, we investigated the effect of pre-treatment with opiate receptor blockers on cannabis antinociception, since the participation of these receptors in the effects of cannabis and its active constituents is still controversial.

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Materials and methods

Animals. Male Sprague-Dawley rats (200–250 g) were used, kept under standardized environmental conditions (room temperature 20±2° C, humidity 60%). Some rats were prepared for intraventricular injection with a cannula implanted in the lateral brain ventricle, as described by Altaffer et al. (1970). For each treatment, groups of ten rats were used.

Drugs and procedure. An extract of *Cannabis sativa L.* was obtained by petroleum ether treatment and contained 14.80% Δ^9-tetrahydrocannabinol (Δ^9-THC) as determined by gas chromatography against a known concentration of decachlorobiphenyl as internal standard. After distillation of the solvent, the residue was suspended in 10% Tween 80 in saline and administered at oral doses of 5 and 15 mg/kg, expressed as Δ^9-THC.

6-Hydroxydopamine HBr (6-OHDA, Hoffmann-La Roche, Basel) was dissolved in 20 μl saline with ascorbic acid (1 mg/ml) and injected intracerebroventricularly twice (24-h interval) at a dose of 150 μg 3 days before the extract in order to cause degeneration of catecholamine terminals and catecholamine depletion (Breese and Traylor 1970).

Naloxone and naltrexone (Endo Lab., Garden City, NY, USA) its analogue with long-lasting activity (Reuning et al. 1979), both relatively selective antagonists of μ-type opiate receptors (Zukin and Zukin 1981), were dissolved in saline and injected at doses of 2 and 10 mg/kg SC 15 min before the cannabis extract; (−)-N-(3-furylmethyl) α-normetazocine methane-sulfonate (MR 1452, kindly supplied by Boehringer Sohn, Ingelheim FRG) and N,N-bisallyl-Tyr-Gly-Gly-Ψ-(CH_2S)-Phe-Leu-OH (ICI 154, 129) purported antagonists of, respectively, κ and δ-type opiate receptors (Ben Sreti and Sewell 1982; Spampinato and Candeletti 1985; Shaw et al. 1982), were injected subcutaneously, at the same doses and time before cannabis. Control animals received the vehicle only. Data were analyzed by Student's *t*-test.

Analgesimetric procedures. The antinociceptive effect was evaluated using the tail flick (D'Amour and Smith 1941) and vocalization (Paalzow et al. 1978) test. In the tail flick test the strength of radiant heat was adjusted so as to obtain a tail flick latency of 3.7±0.15 s in control animals and values were recorded by an automated device; a cut-off time of 10 s was fixed. For the vocalization threshold (measured in mA), two stainless steel 30 gauge electrodes were inserted in the middle section of the tail and electrical stimu-

lation consisted of the application of trains of 1 s duration, containing 125 shocks of 1.6 ms width delivered from a high frequency square wave constant current generator. The maximal intensity of the current delivered was 2 mA.

Individual baseline tail flick latency and vocalization thresholds were determined in three pre-tests 20 min before drug administration.

Results

Effect of 6-OHDA pre-treatment. As shown in Fig. 1, the cannabis extract given to rats at oral doses of 5 and 15 mg/kg Δ^9-THC raised the threshold to nociceptive stimuli of radiant heat (top) and electrical stimulation (bottom). With the higher dose, the cannabis-induced antinociceptive effect reached its peak within 60 min and the pain threshold remained maximal throughout the observation period (3 h). The rise in nociceptive threshold after cannabis extract was significantly ($P<0.01$) lower in animals pre-treated with 6-OHDA. The neurotoxin per se did not significantly affect the pain threshold in the interval before cannabis (data not shown).

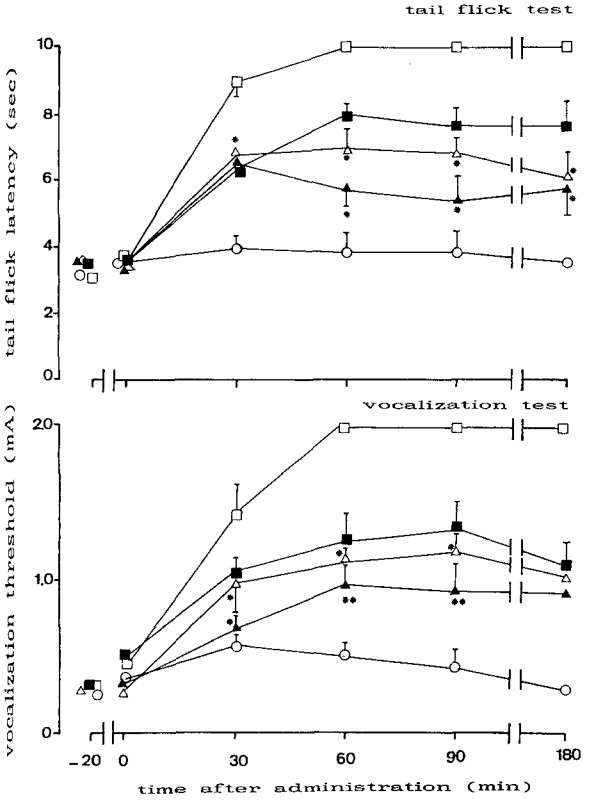

Fig. 1. Effect on nociceptive threshold of a cannabis extract orally administered to rats pretreated or not with 6-hydroxydopamine (6-OHDA). Tail flick latency (*top panel*) and vocalization test (*bottom panel*). (o——o) Vehicle, (■——■) cannabis extract 5 mg/kg (as Δ^9-THC), (□——□) cannabis extract 15 mg/kg (as Δ^9-THC), (▲——▲) cannabis extract 5 mg/kg after 6-OHDA pretreatment, (△——△) cannabis extract 15 mg/kg after 6-OHDA pretreatment. See text for schedule of treatment. M±SEM in ten animals. * $P<0.01$ vs rats treated with cannabis only, at the corresponding dose. ** $P<0.05$ vs rats treated with cannabis only, at the corresponding dose.

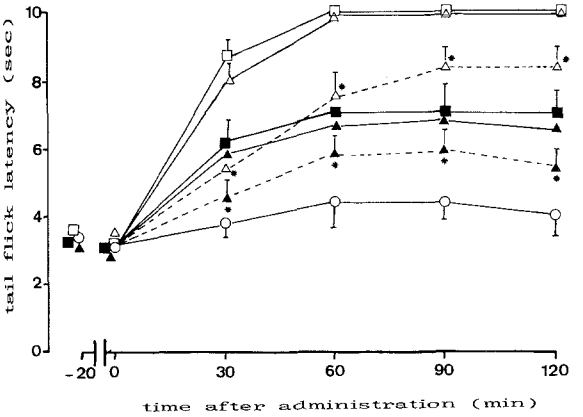

Fig. 2. Tail flick test. Effect on nociceptive threshold of a cannabis extract orally administered to rats pretreated or not with different doses of naloxone. (o——o) Vehicle, (■——■) cannabis extract 5 mg/kg (as Δ^9-THC), (□——□) cannabis extract 15 mg/kg (as Δ^9-THC). Cannabis extract 5 mg/kg after naloxone pretreatment, 2 mg/kg SC (▲——▲) or 10 mg/kg SC (▲----▲). Cannabis extract 15 mg/kg after naloxone pretreatment, 2 mg/kg SC (△——△) or 10 mg/kg SC (△---△). M±SEM in ten animals. * $P<0.01$ vs rats treated with cannabis only, at the corresponding dose

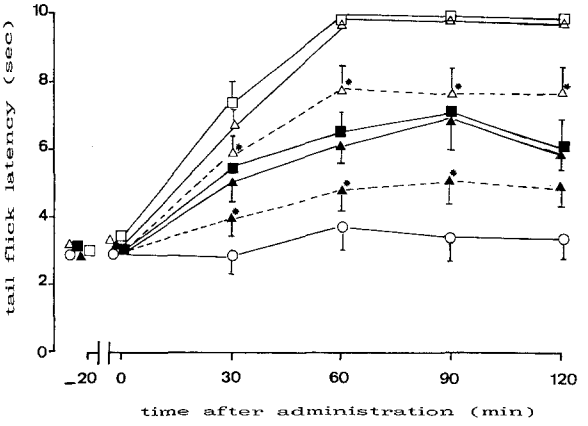

Fig. 3. Tail flick test. Effect on nociceptive threshold of a cannabis extract orally administered to rats pretreated or not with different doses of naltrexone. (o——o) Vehicle, (■——■) cannabis extract 5 mg/kg (as Δ^9-THC), (□——□) cannabis extract 15 mg/kg (as Δ^9-THC). Cannabis extract 5 mg/kg after naltrexone pretreatment, 2 mg/kg SC (▲——▲) or 10 mg/kg SC (▲----▲). Cannabis extract 15 mg/kg after naltrexone pretreatment, 2 mg/kg SC (△——△) or 10 mg/kg SC (△----△). M±SEM in ten animals. * $P<0.01$ vs rats treated with cannabis only, at the corresponding dose

Effect of narcotic antagonist pre-treatment. Naloxone, naltrexone and MR 1452 did not block the antinociceptive effect of the cannabis extract (5 and 15 mg/kg Δ^9-THC) at the dose of 2 mg/kg; only the dose of 10 mg/kg significantly prevented the onset and development of antinociception (Fig. 2, Fig. 3 and Fig. 4 show the action of these narcotic antagonists, ascertained by tail flick test). ICI 154, 129 an antagonist of δ-type opiate receptors, partially prevented the cannabis-induced rise in nociceptive threshold only at the higher dose adopted, 10 mg/kg SC (Fig. 5).

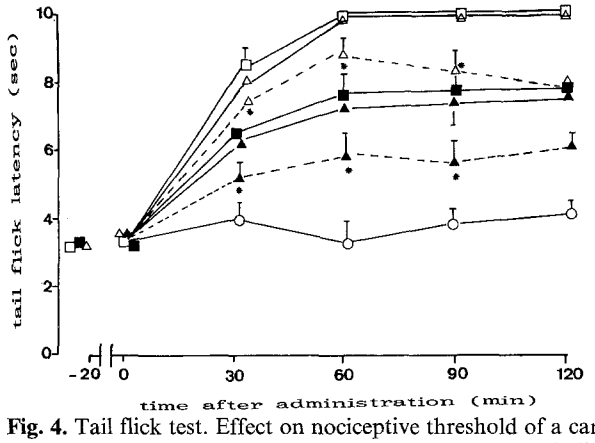

Fig. 4. Tail flick test. Effect on nociceptive threshold of a cannabis extract orally administered to rats pretreated or not with different doses of MR 1452. (○—○) Vehicle, (■—■) cannabis extract 5 mg/kg (as Δ^9-THC), (□—□) cannabis extract 15 mg/kg (as Δ^9-THC). Cannabis extract 5 mg/kg after MR 1452 pretreatment, 2 mg/kg SC (▲—▲) and 10 mg/kg SC (▲---▲). Cannabis extract 15 mg/kg after MR 1452 pretreatment, 2 mg/kg SC (△—△) and 10 mg/kg SC (△---△). M ± SEM in ten animals. * $P<0.01$ vs rats treated with cannabis only, at the corresponding dose

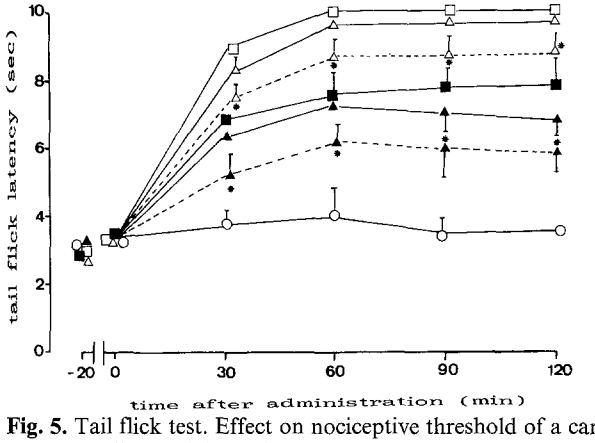

Fig. 5. Tail flick test. Effect on nociceptive threshold of a cannabis extract orally administered to rats pretreated or not with different doses of ICI 154, 129. (○—○) Vehicle, (■—■) cannabis extract 5 mg/kg (as Δ^9-THC), (□—□) cannabis extract 15 mg/kg (as Δ^9-THC). Cannabis extract 5 mg/kg after ICI 154, 129 pretreatment, 2 mg/kg SC (▲—▲) and 10 mg/kg SC (▲---▲). Cannabis extract 15 mg/kg after ICI 154, 129 pretreatment, 2 mg/kg SC (△—△) and 10 mg/kg SC (△---△). M ± SEM in ten animals. * $P<0.01$ vs rats treated with cannabis only, at the corresponding dose

Discussion

It appears from this study that a cannabis extract has lasting antinociceptive activity when given to rats, thus confirming the results of antinociception experiments in this and other animal species with cannabis extracts or its active constituents, particularly Δ^9-THC (Buxbaum 1972; Sofia and Barry 1972; Chesher et al. 1973; Sofia et al. 1973; Sofia et al. 1975; Wilson and May 1975; Bloom et al. 1977; Bloom and Dewey 1978; Tulunay et al. 1981). The vocalization test (electrical tail stimulation) enables us to exclude the possibility that the rise in nociceptive threshold was due merely to motor impairment of the animal through the well-known effect on behaviour elicited by these compounds, previously shown also by us (Ferri et al. 1981)

Cannabis and its derivatives have been the subject of extensive investigations on their relationship with narcotic analgesics. Some reports showed that they potentiate morphine analgesia and that narcotic antagonists naloxone (Wilson and May 1975; Bloom et al. 1977) and chlornaltrexamine (Tulunay et al. 1981) prevent or remove the antinociceptive effect elicited by some cannabinoids. However, there are differences in other studies on the subject: not only do cannabis extracts and pure cannabinoids, including Δ^9-THC, fail to potentiate the antinociceptive effect of another opiate, pethidine, but nalorphine too could not be shown to antagonize the antinociception (or inhibition of gastrointestinal motility) induced by either cannabis or cannabinoids (Chesher et al. 1973; Chesher 1980). Moreover, development of a cross tolerance between opiates and cannabinoids, as regards antinociception, is denied or considered only one-way by some investigators (Bloom et al. 1977; Bloom and Dewey 1978) or acknowledged by others (McMillan et al. 1971). On the other hand, the effectiveness attributed to Δ^9-THC in attenuating naloxone-precipitated withdrawal in morphine-dependent rats and mice (Hine et al. 1975; Bhargava 1976) is not shared by other cannabinoids in morphine-dependent monkeys (Aceto et al. 1975).

Our findings do not fully support studies suggesting common receptor binding sites for cannabinoids (natural components of cannabis) and opiates, at least for μ and δ-type opiate receptors.

In actual fact, both naloxone and naltrexone, which are preferential μ-receptor antagonists (Zukin and Zukin 1981) completely failed to prevent the rise of nociceptive threshold induced by cannabis at a dose fully effective in preventing similar effects of an opiate. Similarly, the lack of effect of 2 mg/kg MR 1452 enables us to exclude a cannabis interaction with κ-type opiate receptors, due to the relative selectivity of this chemical for this type of receptors (Ben Sreti and Sewell 1982; Spampinato and Candeletti 1985). At best, considering the partial effect of a dose of 10 mg/kg of antagonists, μ and κ opiate receptors can be allowed only a marginal role in cannabis antinociception. Problems emerge only as regards δ-opiate receptors considering the partial prevention of cannabis antinociception by ICI 154, 129 at a dose (10 mg/kg) assumed by some authors (Cowan and Gmerek 1982) to be relatively low for the range of activity of this antagonist. The involvement of at least one sub-type of opiate receptors cannot be completely excluded in the action of cannabis, however more information could come from new antagonists endowed with higher selectivity and potency against the complex population of opiate receptors. At present, in interpreting the phenomenon of cannabis-induced antinociception the results concerning pathways distal to opiate systems appear more significant. Since 6-OHDA injected into the lateral ventricle of the brain leads to a selective degeneration of catecholamine-containing nerve terminals (Breese and Traylor 1970), one might suggest that catecholaminergic pathways participate in the mediation of cannabis antinociception. The earliest reports attempted to establish a connection between biogenic amines in the CNS and the effects of cannabis and its active constituents. However, this relationship is not yet firmly established. As regards catecholamines, the data in the literature on their endogenous concentrations and turnover after cannabis and cannabinoid administration are conflicting; some investigators reporting an increase in the synthesis and turnover of dopamine and noradrenaline in rat and mouse brain, with little or no change in their endogenous levels (Maitre et al. 1970; Bloom et al. 1978; Bloom 1982) while

other investigators have not detected any changes in either levels or turnover of both catecholamines (Bracs et al. 1975).

The participation of catecholamines in the central effects of cannabis, including antinociception, is supported by the present findings and others reported previously (Ferri et al. 1981) concerning the modifications induced by 6-OHDA in the behavioral response of the rat after administration of an extract of cannabis rich in Δ^9-THC. On the other hand, many studies indicate that catecholaminergic pathways play a role in antinociception elicited by different classes of drugs; for instance, manipulation of catecholamine levels by central administration of the same neurotoxin 6-OHDA reduced morphine antinociception (Ayhan 1972; Friedler et al. 1972; Bläsig et al. 1973) as well as that induced by mescaline (Ferri et al. 1977) another hallucinogenic drug.

We believe that the participation of opiate pathways in cannabis antinociception is not fundamental, although we cannot exclude that multilple factors may be involved in the development of the phenomenon, as for other drugs. Recently, interactions have been shown between cannabinoids and adrenergic (Hillard and Bloom 1982) or dopaminergic (Bloom 1984) receptors that could be the result of changes caused in physical properties of cell membrane: if this is the case, an alteration of the neural membrane environment could, evidently, affect the properties and the functions of several receptors.

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