Effects of anti-acetylcholine drugs on aggressive behaviour induced by *Cannabis sativa* in REM sleep-deprived rats

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Cannabis sativa extracts (CE) and (—)-Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC) induce aggressive behaviour in paired rats previously deprived of rapid eye movement (REM) sleep (Alves, Goyos & Carlini, 1973; Carlini, 1977). We have examined the influence of pretreatment with anti-acetylcholine drugs on agressive behaviour induced by CE in REM sleep-deprived rats.

Genetically similar female Wistar rats, after weaning at 30 days, were randomly housed in pairs in wire cages (16 × 30 × 18 cm) at 22 ± 1°, on a non-reversed 12-h-light cycle, animals were 90 days old at the beginning of the experiments and had free access to food and water. CE was suspended in 0·9% NaCl + Tween-80 (Carlini & Kramer, 1965). Analysis of the CE showed it to contain 68% of Δ^9-THC, 5% of cannabinol and 1% of cannabidiol. Experiments were replicated seven times. All rats in each replication were REM-deprived three times, with an intersession period of 16 days, during which they were kept in pairs in their home cages. In each session REM sleep-deprivation was performed according to Alves & others (1973). After 96 h of REM deprivation, the animals were removed from the 6 cm platforms in water containers in fixed numerical order and returned to their home cages. About 10 min after the end of each of the three sessions of REM-deprivation all rats were injected with a dose of CE (20 mg kg^{-1}, i.p.) and were observed for aggressive behaviour for 100 min. This dose of CE had previously produced more than 2 × 10^3 s of fighting in all pairs of REM sleep-deprived rats tested. 1 h before the end of REM-deprivation of both 2nd and 3rd sessions anti-acetylcholine pretreatment was given. This consisted of intraperitoneal injections of atropine sulphate (25, 50, 100 mg kg^{-1}), scopolamine hydrobromide (10, 20 mg kg^{-1} or atropine methyl nitrate (50 mg kg^{-1}). All dosages refer to the salt, and all drugs were dissolved in distilled water. Controls received only the vehicle (1·0 ml kg^{-1}).

The same pretreatment was given in both the 2nd and 3rd sessions within a group. Aggressive behaviour was recorded in seconds by two different observers as described by (Palermo Neto, Nunes & Carvalho, 1975). This criterion is very objective since highly positive correlations ($r = +0.892$ to $+0.965$) were found between the scores from the two observers. To minimize possible effects of circadian changes that could interfere with the results, the experiments were designed to allow all subjects to be observed at the same time of the day, i.e., at 9.30 a.m. The results were analysed using the Mann–Whitney U one-tailed test for differences in duration of aggressiveness among the several groups. Differences were considered significant when $P < 0.05$.

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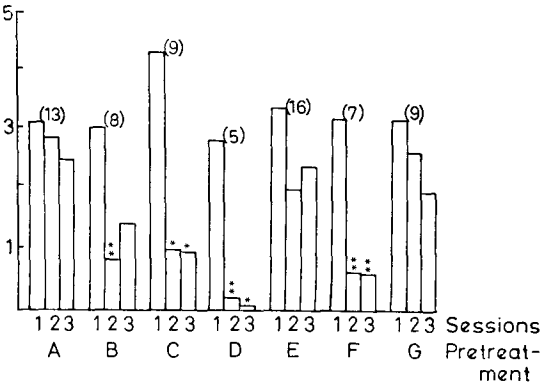

FIG. 1. Influence of anti-acetylcholine drug pretreatment on aggressive behaviour induced by 20 mg kg^{-1} of *Cannabis sativa* extract in REM sleep-deprived rats. The letters under the columns mean pretreatment of the animals 1 h before the end of both 2nd and 3rd sessions with 1·0 ml kg^{-1} of distilled water (group A); 25, 50 and 100 mg kg^{-1} of atropine sulphate (groups B, C and D respectively); 10 and 20 mg kg^{-1} of scopolamine hydrobromide (groups E and F respectively) and 50 mg kg^{-1} of atropine methyl nitrate (group G). Numbers in brackets correspond to the number of pairs used in each group. Asterisks mean statistically significant difference between that session and the first within a group, respectively at * $P < 0.01$ and ** $P < 0.05$ levels (U test). Ordinate: Aggressive behaviour (s × 10^3).

The results are outlined in Fig. 1. Irritability, hyperactivity and aggressiveness appeared in all pairs of 96 h REM sleep-deprived rats treated with 20 mg kg^{-1} of CE (1st session of groups A–G). The aggressive behaviour did not differ significantly either among the 1st sessions of all groups or among the 3 consecutive sessions within the control group (group A). Thus, any alteration in intensity of fighting between the 2nd or 3rd sessions and the first in the remaining groups would be due to an effect of drug pretreatment. Animals pretreated with 25, 50 or 100 mg kg^{-1} of atropine sulphate (groups B, C and D respectively) fought significantly less than after CE treatment alone. This decrease in aggressive behaviour seems to be dose related but only the 50 and 100 mg kg^{-1} doses of atropine sulphate were able to diminish aggressiveness in both the 2nd and 3rd sessions. In this respect, the fighting behaviour was absent in animals pretreated with 100 mg kg^{-1} of

atropine sulphate in the 3rd session (group D). With scopolamine pretreatment (groups E and F) only the larger dose of scopolamine (20 mg kg^{-1}—group F) decreased aggressive behaviour. Atropine methyl nitrate was not able to alter fighting (group G). The reason seems obvious: it does not cross the blood-brain barrier. This fact strongly suggests a central action for the former drugs. It appears reasonable to assume this since pretreatment of rats with 20 mg kg^{-1} of scopolamine (group F), a tertiary amine that readily crosses blood-brain barrier, decreases aggressiveness to the the same extent as 50 mg kg^{-1} of atropine sulphate (group C), a quaternary amine that penetrates less promptly into the central nervous system. Taken together, our results suggest that large doses of anti-acetylcholine drugs decrease aggressiveness induced by CE in REM sleep-deprived rats, probably through a central action.

Several studies support the idea that arousal is mediated, at least in part, by cholinergic mechanisms (De Feudis, 1974). It is well known that large systemic doses of anti-acetylcholine drugs block neocortical activation elicited by several different stimuli or by drugs (Domino & Hudson, 1958; White & Daigneault, 1959). Aggressive behaviour produced by ventromedial hypothalamic stimulation is enhanced by simultaneous stimulation of the midbrain reticular formation (Sheard & Flynn, 1967). This implies that an aroused animal will respond faster and more intensely to the stimuli eliciting aggressiveness than will an unaroused animal. Thus, the evidence presented here favours the conclusion that a decrease in behavioural arousal produced by anti-acetylcholine drugs is responsible for the reduction in aggressive behaviour induced by CE administration in REM sleep-deprived rats. Nevertheless, there is some evidence that dopaminergic neurons are under a cholinergic influence (Głowinski, 1975; Agid, Guyenet & others, 1975) and that dopaminergic systems are involved in aggressive behaviour (Rolinski, 1973; Senault, 1974). Palermo Neto & Carlini (1972) observed that pretreatment of rats with L-dopa potentiates the ability of CE to induce aggressive behaviour in starved rats. On the other hand it is known that high concentrations of acetylcholine drugs in the brain cause non-specific blockade of several central receptors, including the dopamine receptor (Zebrowska-Lupina, Zdzislaw & Zbigniew, 1975). These findings suggest that our results may also stem from either an unbalance between cholinergic-dopaminergic systems within the brain or a non-specific blockade of central dopamine receptors at the high doses used.

This research was supported by a fellowship (No. 75/569 and 75/569-R) from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) to H. de Souza. The authors wish to express sincere thanks to Dr E. A. Carlini of the Depto. de Psicobiologia, Escola Paulista de Medicina for both *Cannabis sativa* extract and atropine methyl nitrate supplies.

March 16, 1978

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