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BRIEF COMMUNICATION

Tetrahydrocannabinol and Acetylcholinesterase[1]

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MOSS, D. E., P. L. PECK AND R. SALOME. *Tetrahydrocannabinol and acetylcholinesterase.* PHARMAC. BIOCHEM. BEHAV. 8(6) 763–765, 1978. – Recent evidence suggests that the psychoactive effect of delta-9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive constituent of marihuana, may be mediated through an alteration of cholinergic neurotransmission. One possible mechanism by which Δ^9-THC could have its effect is by affecting acetylcholinesterase (AChE) and there is evidence that has suggested that this may be an important mechanism. The results reported in the present study have shown that there is no physiologically important interaction between AChE and Δ^9-THC or its metabolites that could explain its psychoactive effects or the profound clinical depression observed when human marihuana users are administered the cholinesterase inhibitor physostigmine.

Tetrahydrocannabinol Acetylcholinesterase Marihuana Physostigmine Depression

STUDIES of the effects of delta-9-tetrahydrocannabinol (Δ^9-THC), the psychoactive component in marihuana [10,15], have shown that this drug affects brain serotonin [8, 18, 19, 21], norepinephrine [8], and dopamine metabolism [7,9]. However, other recent evidence suggests that the psychoactive effects of this drug might be more directly related to cholinergic activity. Specifically, El-Yousef, Janowsky, Davis and Rosenblatt [6] found that the administration of physostigmine, a cholinesterase inhibitor, to two marihuana intoxicated individuals resulted in a sudden profound depression. In addition, noticeable improvement in the marihuana/physostigmine depression occurred within 1 min of the administration of atropine, a muscarinic cholinergic receptor antagonist. One of the main criticisms of the clinical observations of El-Yousef *et al.* [6] is that there was no control for the direct effects of physostigmine on mood and it is well known that physostigmine has direct effects of this nature [12,22]. The significance of the physostigmine/marihuana depression observed by El-Yousef *et al.* [6] has, however, been established by serendipitous observations by Davis, Hollister, Overall, Johnson and Train [4] who administered physostigmine to normal humans to study its effects on cognition and affect. The dose of physostigmine used in their study had no significant effect on mood as compared to a placebo control group, however, one subject who later admitted to daily marihuana use became depressed and reported being "more depressed than I've ever felt" during the following 72 hr. This report corroborates the observations of El-Yousef *et al.* [6] concerning the profound depression produced by an interaction between physostigmine and marihuana and, furthermore, demonstrated that the depression is not due to the effect of physostigmine alone insofar as many other subjects received the same dose of physostigmine and did not differ from placebo controls. These observations, while not eliminating possible interactions with other neurotransmitter systems, have suggested that cholinergic neurotransmission may be important in the regulation of affect, probably in conjunction with the catecholamines [11]. Therefore, a clear understanding of the brain mechanisms by which Δ^9-THC produces its psychoactive effect would not only resolve important questions regarding the pharmacological effects of marihuana but, more importantly, it would provide new insights into the neurochemical functions involved in the control of affect and depressions that continue to be resistant to traditional therapy [20].

An obvious hypothesis that would explain the observed marihuana/physostigmine depression would be that Δ^9-THC or its metabolites have some direct effect on acetylcholinesterase (AChE) that would change the activity of this enzyme in the synapses of the central nervous system during marihuana intoxication and greatly potentiate the interaction between AChE and physostigmine which would produce the observed depression. An interaction between THC and AChE has, in fact, been suggested by

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Brown [3] based on behavioral data. More direct evidence, however, comes from the report by Askew, Kimball and Ho [2] who determined that Δ^9-THC caused a small, statistically significant but pharmacologically insignificant, decrease in rat brain AChE 1 hr after 5 mg/kg given IV. However, Yoshimura, Fujiwara, and Ueki [24] found no effect on AChE after IP injection of 6 mg/kg Δ^9-THC. Although these results show that the direct effect of THC on AChE may be of questionable significance, Rosenblatt, Janowsky, Davis and El-Yousef [17] demonstrated that pretreatment with THC produced a highly significant potentiation of physostigmine toxicity in vivo. These results suggest that there may be an interaction between THC and physostigmine which produces a greatly potentiated inhibition of AChE. Such an interaction would explain the potentiated physostigmine toxicity by THC as reported by Rosenblatt *et al.* [17] and the physostigmine/marihuana depression reported by El-Yousef *et al.* [6] and Davis *et al.* [4].

Preliminary experiments conducted in this laboratory (unpublished) have shown that Δ^9-THC added to AChE assays in vitro in concentrations up to 5 mg/ml has no detectable effect on enzyme interactions with its substrate (i.e., no direct inhibition) or with physostigmine as an inhibitor. However, because of the considerable amount of evidence that the in vivo psychoactive effect of Δ^9-THC depends upon metabolic conversion of the drug to various metabolities, particularly to 11-hydroxy-Δ^9-THC [13,23], it was not surprising that Δ^9-THC added in vitro had no effect. The purpose of the present experiment was to extend these preliminary results and determine if an interaction between AChE, physostigmine, and in vivo metabolites of Δ^9-THC existed under physiological conditions.

MATERIALS AND METHOD

Drug Administrations

Δ^9-THC was administered in vivo to allow for metabolic conversion of the drug and distribution of the metabolites into nervous tissues under physiological conditions. The Δ^9-THC was administered to 10 rats as a single 20 mg/kg dose by forcing a tube down the esophagus for intragastric injection. The drug was prepared by 1:10 dilution into olive oil of the 200 mg/ml solution in ethanol supplied by the Research Technology Branch of NIDA so that the volume administered to all rats was 1 ml/kg. Ten control rats were administered an equal volume of olive oil containing 10% ethanol. Physostigmine salicylate was administered to five rats from each of the above described groups in a dose of 0.2 mg/kg (IP) 3 hr and 45 min after the THC and 15 min before decapitation for AChE assays. Rectal temperatures were monitored by a rectal thermister probe inserted 6 cm to determine if the dose of Δ^9-THC given was pharmacologically active. The single intragastric administration of 20 mg/kg Δ^9-THC produced a highly significant drop in rectal temperature. The animals injected with THC had a mean pretreatment temperature of 37.44° (±0.2°) while the controls had a virtually identical mean temperature of 37.47° (±0.2°). Four hr later, however, the THC treated animals had a mean temperature of only 34.80° (±0.24°) while the controls remained unchanged at 37.35° (±0.11°) The ambient temperature was between 20 and 21°. This large decrease in rectal temperature is much greater than that observed by Abel [1] who injected THC intramuscularly. The difference is probably the result of excellent absorption, metabolism and distribution of the drug after intragastric injection.

Assay of Acetylcholinesterase

Fifteen min after the injection of physostigmine or a control injection of vehicle alone, the animals were decapitated for in vitro assay of AChE activity. The in vitro assays were conducted according to the procedure of Ellman, Courtney, Andres and Featherstone [5] at 25.0°, pH 7.0, with membrane bound AChE from the rat brains prepared according to the methods of Moss and Fahrney [16]. All assays were completed within 20 min of death and consisted of triplicate assays of activity at 0.5 mM acetylthiocholine substrate. Because physostigmine forms a stable covalent complex with AChE during the inhibition of this enzyme, inhibition produced in vivo disappears slowly with half-times reported from about 30 min to 1 hr after death [14] and even persists through the homogenization of the tissue and dilutions required for the assay of residual activity. Therefore, insofar as the assays were completed within 20 min of death, the inhibition observed is a good estimate of actual in vivo inhibition produced under the various drug conditions.

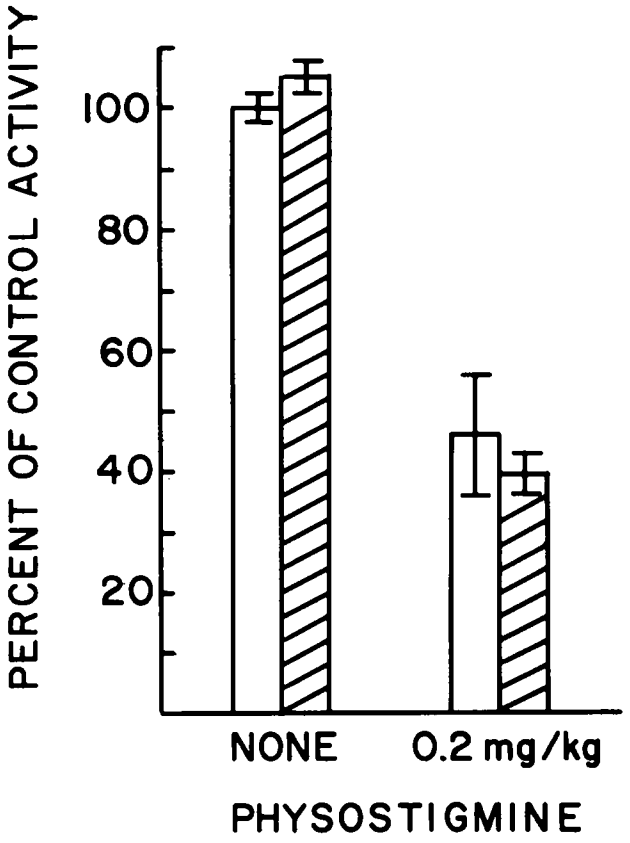

FIG. 1. AChE activity as affected by Δ^9-THC and physostigmine. Calculations of all percent values are based on the activity level of the control condition (no physostigmine, no Δ^9-THC) as 100%. The AChE activity from animals treated with 20 mg/kg Δ^9-THC is shown with shaded bars whereas activity from control animals (no Δ^9-THC) is shown without shading. Each group contained five animals. Error bars represent one standard error of the mean.

RESULTS

The results of this experiment are shown in Fig. 1. Analysis of variance computed from these data showed that the main effect of Δ^9-THC treatment was not significant, F = 0.02, the interaction between THC and physostigmine was not significant, F = 1.01, and, as expected, the main effect of physostigmine alone was highly significant, F = 100.9, $p<0.001$.

DISCUSSION

These results seem to eliminate any realistic possibility of a pharmacologically important interaction between THC and physostigmine that can be attributed to any effects on AChE. These results leave two major findings unexplained. Specifically, the pharmacological basis for the observed interaction between THC and physostigmine that controls mood and depression [4,16] and the large potentiation of physostigmine toxicity by pretreatment with THC [17] have not been adequately explained. It seems reasonable that some pharmacological effect of physostigmine, other than the inhibition of AChE, may interact with THC in such a way as to produce depression. Systematic studies of other possible interactions between THC and physostigmine will certainly lead to better understanding of the neurochemical control of mood and depressions.

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