Antibacterial activity of Δ^9-tetrahydrocannabinol and cannabidiol

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The minimum inhibiting concentrations (MIC) of Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) for staphylococci and streptococci in broth are in the range of 1–5 μg/ml. In the same range, both compounds are also bactericidal. In media containing 4% serum or 5% blood the antibacterial activity is strongly reduced (MIC 50 μg/ml). Gram-negative bacteria are resistant to THC and CBD.

INTRODUCTION

Extracts of *Cannabis sativa* (marihuana, hashish) do not only influence human and animal behaviour, but also display an antibacterial action on gram-positive bacteria (Kabelik, 1957; Krejči, 1958; Ferenczy, Gracza and Jacobey, 1958; Schultz and Haffner, 1959). Most observations on this action however have been made with crude extracts or with only partly purified unidentified substances isolated from such extracts. The present report pertains to quantitative determinations of the bacteriostatic and bactericidal action of purified Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) which are the main constituents of cannabis.

MATERIALS AND METHODS

Drugs. Δ^9-trans-tetrahydrocannabinol (batch UNC 332) was obtained from the Bureau of Narcotic Drugs of the United Nations. Cannabidiol was isolated by Dr. F. J. Küppers, Laboratory of Organic Chemistry, University of Utrecht. Both drugs were dissolved in ethanol 70% to a concentration of 2 mg/ml. From this stock solution, dilutions were made in saline.

Strains. Staphylococcus aureus ATCC 6538; laboratory strains of staphylococci, streptococci and Enterobacteriaceae.

Media. Nutrient broth agar pH 7.4 (RIV). Horse blood agar prepared by adding 5% defibrinated horse blood to nutrient broth agar pH 7.4 (RIV).

Antibacterial tests. Two methods were employed to estimate the antibacterial activity of THC and CBD.

1. Minimum inhibitory concentrations (MIC's) were determined as follows. Agar media containing increasing concentrations of the drugs were inoculated with suspensions of the test organisms in saline by means of a Steers replicator (Steers, Folts and Graves, 1959), the inoculum being approximately 10^3 cells of each of the test organisms. MIC's were read after incubation overnight at 37 C.
2. To investigate whether the action of both substances is primarily bacteriostatic or bactericidal, suspensions of *Staphylococcus aureus* ATCC 6538 were prepared in saline (without and with 4% horse serum) containing appropriate concentrations of THC or CBD. The mixtures were placed in a waterbath at 20 C. At regular time intervals the number of living cells per ml were determined by a standard pour-plate method. The bactericidal effect is expressed as the number of decimals reduction of viable cells.

RESULTS

Minimum inhibitory concentrations are listed in Table 1. THC and CBD were significantly active only against the gram-positive bacteria tested. The MIC's on horse blood agar were at least ten times higher than those on nutrient broth agar.

The results of the tests for the determination of bactericidal activity, using *S. aureus* as the test organism, are summarized in Table 2. 4% horse serum when added to the saline made both THC and CBD ten times less active. These data agree with the results given in Table 1.

Table 1.

Organism	Number of strains	M.I.C. (μg/ml)			
		Nutrient broth agar		Horse blood agar	
		THC	CBD	THC	CBD
Staphylococcus aureus	4	2–5	1–5	20–50	20–50
Streptococcus pyogenes	1	5	2	50	50
Streptococcus milleri	1	2	1	50	50
Streptococcus faecalis	1	5	5		
Escherichia coli	4	>100	>100		
Salmonella typhi	1	>100	>100		
Proteus vulgaris	1	>100	>100		

Table 2. Number of decimals reduction after 1 h and 4 h exposure at 20C.

		Control (without THC or CBD)	THC (μg/ml)					CBD (μg/ml)				
			2	5	10	20	50	2	5	10	20	50
Saline	1 h	<0.1	1.2	1.3	2.6			2.5	2.8	3.1		
	4 h	<0.1	>4	>4	>5			>5	>5	>5		
Saline + 4% serum	1 h	<0.1			<0.1	<0.1	0.2			<0.1	0.1	2.6
	4 h	<0.1			0.5	0.8	2.7			0.1	1.1	3.2

DISCUSSION

Tables 1 and 2 show that at similar levels of concentration both THC and CBD are bacteriostatic as well as bactericidal. The lower activity in the presence of 4% horse serum or 5% horse blood may be due to protein binding a large proportion of either substance (Garrett and Hunt, 1974). Our findings are in agreement with those of Krejči (1958). Starting with a petroleum ether extract of *Cannabis sativa* var. *indica* this author isolated an amorphic substance. Ten μg/ml of this substance dissolved in peptone water caused about a hundredfold reduction in viable cells of *S. aureus* after 4 hours incubation at 37 C. For a bactericidal effect in peptone water containing 10% blood or 10% blood plasma, about 100 times higher concentrations were needed. Exact quantitative comparisons with our data cannot be made as in Krejči's preparations the THC or CBD concentrations could not be determined. Moreover, the antibiotic action of Cannabis extracts may be due to other cannabinoids as well. Gal, Vayda and Bekes (1969) showed that cannabidiolic acid is also antibacterially active.

Both THC and CBD are poor antibacterial substances in the presence of serum; besides they disappear quickly from the blood (Agurell et al., 1970). Therefore, they probably will never be used as therapeutics. One sensible application might be in preparations for topical use. As food preservatives they may not be taken into consideration because of the many other pharmacological activities, especially of THC.

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