B). On the contrary, addition of cAMP without exogenous PK stimulated phosphorylation almost to the same extent as cAMP and PK together (Figure A). This finding may serve as evidence for sufficiently high endogenous PK activity in our preparations.

The cAMP concentration responsible for the half maximal stimulation of the Mg^{2+}-dependent phosphorylation of cardiac SR in the controls in vitro amounted to 0.15×10^{-6} *M*. The corresponding increase of phosphorylation represented the 2.5-fold of the basic Mg^{2+}-dependent value (Figure A).

In preparations from hearts pretreated with ISO in vivo, phosphorylation without addition of cAMP or PK exceeded by 30% the extent of the half maximal stimulation reached with cAMP in the controls in vitro. However, this phosphorylation would represent not only the basic Mg^{2+} but, to a certain extent, also a cAMP-dependent incorporation of ^{32}P into phosphoserine[18].

However, subsequent addition of 0.15×10^{-6} cAMP to preparations from ISO-treated hearts increased phosphorylation by a further 8% only (Figure B). This poor response to exogenous cAMP suggests a stimulatory effect on the cAMP-producing systems obtained already by the application of ISO in vivo. The latter stimulatory effect corresponds to an amount of cAMP produced, which would stimulate the phosphorylation obtained by in vitro application of cAMP to the controls, approximately by 74% of the maximal stimulation (Figure A).

The present results appear to confirm the validity of our supposition concerning the mechanism of regulating the SR calcium transport by catecholamines (isoproterenol) both in vivo and in vitro.

Zusammenfassung. Die cAMP-abhängige Inkorporation von ^{32}P in das sarkoplasmatische Reticulum (SR) wurde im Zusammenhang mit Ca-Akkumulation in Kontrollherzen und in Herzen 2 h nach in vivo Verabreichung von 7,5 mg/kg Isoproterenol gemessen und gezeigt, dass die Katecholamine den intrazellulären Kalziumtransport in vitro sowie in vivo über die cAMP-stimulierte und PK-abhängige Phosphorylierung einer Proteinfraktion des SR regulieren.

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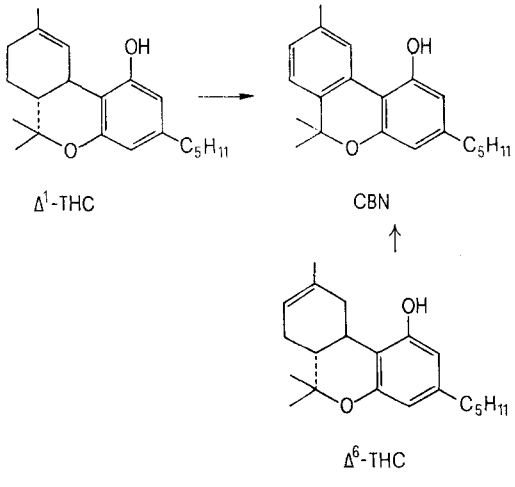
Cannabinol: a Rapidly Formed Metabolite of Δ^1- and Δ^6-Tetrahydrocannabinol

The primary metabolic pathway of Δ^1-tetrahydrocannabinol (Δ^1-THC) and Δ^6-THC is considered to be a mono-oxygenation, mostly at allylic positions[1]. Recently, several indications that cannabinol (CBN) and its oxidized derivatives may be Δ^1-THC metabolites have also been reported. Relatively large amounts of CBN were found[2] by one of us (N. McC.) in the blood of Δ^1-THC smokers. Later, WIDMAN et al.[3] observed high concentrations of CBN relative to Δ^1-THC (but in less than 0.1% overall yield) in rat bile after the i.v. injection of Δ^1-THC. BEN ZVI et al.[4] have isolated CBN-7-oic acid from the urine of rhesus monkeys administered with Δ^1-THC.

It has been shown[5] with rats that the presence of CBN in administered Δ^1-THC causes an increased rate of disappearance of Δ^1-THC from the blood. This results in higher levels of CBN relative to Δ^1-THC than would be expected from the metabolism of the pure compounds. The possibility that both MCCALLUM[2] and WIDMAN et al.[3] could have been observing a similar phenomenon, combined with the fact that the amount of CBN determined in blood by both these authors was less than the amount of CBN administered as an impurity in the Δ^1-THC, prompted us to do a careful re-evaluation of CBN as a possible Δ^1-THC metabolite.



Methods and materials. Male rats were injected with the cannabinoid (in 5–10 µl propylene glycol) via a tail vein. Their blood was collected and mixed with heparin and the internal standard[6]; it was then extracted with light petroleum, acidified (1 *N* HCl), reextracted and centrifuged[2]. The petroleum fraction was analyzed by gas chromatography (GLC) (glass column filled with 4% SE 30 on Gas Chrom Q 100 mesh, at 200°, with 60 ml/min nitrogen carrier gas using flame ionization detection). Calibration

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[6] In the Δ^1-THC determinations iso-hexahydro-cannabinol (3,4,5,6-tetrahydro-7-hydroxy-2-methyl-5-isopropyl-9-pentyl-2,6-methane-2H-1-benzoxocin), prepared according to the method of Y. GAONI and R. MECHOULAM, Israel J. Chem. *6*, 589 (1968), was used. Subsequent determinations were done using cannabidiol (which had the same retention time) as internal standard.

curves for the peak height of internal standard compared to peak heights of known amounts of cannabinoids treated according to the above extraction procedure, were constructed and found to be linear. Concentrations of cannabinoids in the rat blood were calculated using the ratios of peak heights.

The ^{14}C-Δ^1-THC (6 mg; ca 8×10^6 dpm) was administered to 2 rats which were killed and exsanguinated after 40 sec. Nonradioactive CBN (ca. 1 mg) was added to the light petroleum before extraction. Thin layer chromatography (TLC) of the extracts gave an excellent separation of Δ^1-THC and CBN which were eluted from the silica gel with ether. CBN (ca. 30 mg) was added to the ^{14}C-CBN eluant and the concentrate was acetylated using pyridine and acetic anhydride. The CBN acetate was recrystallized (m.p. 76.5° from pentane) to constant specific activity (16.3×10^3 dpm/mM).

Nonradioactive Δ^1-THC and Δ^6-THC were purified by TLC and contained 0.1% CBN (by GLC). Purification of ^{14}C-Δ^1-THC was done using dimethylformamide saturated silica gel plates[7]. The ^{14}C-CBN was at the limits of detection (radioactive scan) and appeared to be considerably less than 0.1% of ^{14}C-Δ^1-THC content.

Results and discussion. CBN was identified as a metabolite of Δ^1-THC and Δ^6-THC by GLC and TLC comparison with authentic natural CBN, as well as by the crystallization of metabolic ^{14}C-CBN (as its acetate) to constant specific activity. The latter method has not hitherto been used for the identification of cannabinoid metabolites, although it is widely used in other fields.

CBN is formed from both Δ^1-THC and Δ^6-THC very rapidly; the peak value of CBN is reached 40 sec after the THC administration (see Figure). Δ^1-THC itself peaks at 35 sec. This small difference has been reproduced in 6 series of rats and stresses the rapid nature of the process. Injections of either 1,2 or 20 mg Δ^1-THC gave similar peak yields of CBN relative to the Δ^1-THC concentrations.

That the CBN observed in the blood is a metabolite and does not originate from CBN present in the administered THC is shown by estimation of the amounts of CBN produced. The total amount of CBN administered to a single rat was not more than 1 µg, when the dose of Δ^1-THC was 1 mg. Presumably this amount of CBN will not remain in the blood for 40 sec due to fast distribution. By comparison, the amount of Δ^1-THC in the blood after the same period is only ca. 4% of the amount administered. Yet, we actually established the presence of a total of 2 µg of CBN in the blood after 40 sec. In control experiments, addition of the same amount of Δ^1-THC or Δ^6-THC to fresh rat blood, followed by the usual extraction did not yield detectable amounts of CBN. Δ^6-THC, which in contrast to Δ^1-THC is stable towards chemical oxidation into CBN, also yields CBN when administered to the rat which emphasises the point that the CBN cannot be an artefact of the analytical procedure. The mechanism of the above metabolic dehydrogenation is not clear.

The peak concentration of CBN in the blood (on administration of 1 mg Δ^1-THC) is ca. 0.3 µg/ml and this represents ca. 4% of the amount of Δ^1-THC present at this time. In parallel Δ^6-THC experiments the amount of CBN is higher (ca. 10%) although the disappearance curves of Δ^1-THC and Δ^6-THC are very similar.

The above findings may have significance as regards the mode of action of cannabis. Cannabinol does not produce 'cannabis-type' effects in either humans[8] or monkeys[9] on i.v. injection. However, when administered to humans by i.v. infusion, it shows activity, similar in type, but less than one sixth that of Δ^1-THC[10]. The equilibrium blood levels of CBN in these experiments are not known but it is conceivable that metabolic formation of CBN may represent a form of 'internal infusion' at sufficiently high levels to contribute to the overall intoxication. Furthermore, CBN has been shown to effect changes in the rate of Δ^1-THC metabolism[5]. The consequences of the metabolic production of CBN should be examined in the light of these findings[11].

Zusammenfassung. Cannabinol (CBN) ist als Metabolit des Δ^1-Tetrahydrocannabinols und des Δ^6-Tetrahydrocannabinos im Rattenblut erwiesen worden. Die Gipfel-Konzentration von CBN wird 40 sec nach der i.v. Injektion erreicht. Der mögliche Beitrag von CBN zum Cannabis-Effekt wird diskutiert.

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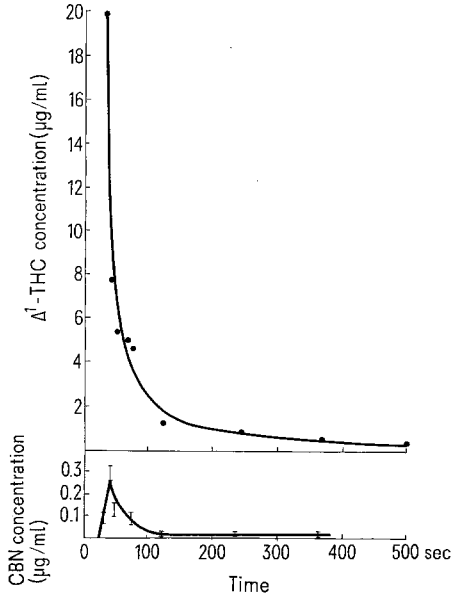

Canabinoid concentrations in rat blood after i.v. injection of 1 mg Δ^1-THC.

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