Table III. Solubilization of succinate dehydrogenase by KCN in the presence of various effectors

Conditions	Extracted PBF (%)
No addition	100
Succinate, 40 m*M*	0
NADH, 5 m*M*	43
IDP, 5 m*M*	21

The Keilin-Hartree heart muscle preparations were pretreated with each effector for 15 min at 30 °C (in the samples with NADH and succinate, 1 m*M* KCN was also added). Other conditions as in the Figure.

period may be interpreted as resulting from a preferential interaction of CN^- with one of the two forms of the enzyme.

In relation to these findings, the effect of KCN on the butanol-solubilized succinate dehydrogenase was investigated. The enzyme was incubated with KCN in various conditions; KCl was added to the control to yield the same ionic strength. The activity was measured at different times without and with full activation by succinate (Table II). In the latter case, no substantial difference between controls and KCN-treated samples is observed, whether incubations are carried out under nitrogen or in air. No protection against irreversible inactivation is then afforded by KCN. On the other hand, if activation by succinate is not performed, the activity in the KCN-treated samples always remains higher in the various conditions tested. These results suggest that KCN partially preserves succinate dehydrogenase against spontaneous deactivation. We shall recall that the flavoprotein solubilized by KCN[6] shows a lower degree of deactivation, as activity remains constant with time and succinate enhances this activity 1.4 times only.

These data suggest a preferential interaction of CN^- with activated succinate dehydrogenase. The lag period thus describes a situation of stationary state for the activated form, being the solubilized enzyme replaced by conversion of the deactivated one. However, effectors which are well known for their activating action on the particulate enzyme[1], prevent to different extents the resolution of succinate dehydrogenase from the membrane by cyanide (Table III).

In our previous work[6] we have shown that CN^- binds to the non-haem iron of succinate dehydrogenase. We may thus conclude that the conversion of the enzyme from a deactivated into an activated state results in an exposure of the non-haem iron-labile sulphide chromophore. Nevertheless, interaction of the enzyme with effectors causes a hindrance at the site of CN^- binding. As to the mechanism of activation, the present data are more consistent with the scheme proposed by McDonald-Gibson and Thorn[4] which includes a free equilibrium between a deactivated and an activated form of succinate dehydrogenase, i.e. the process of activation does not necessarily occur through the binding of an activator[10].

Riassunto. Analizzando l'effetto del cianuro sulla Succinato Deidrogenasi, si conclude che si ha una interazione preferenziale del CN^- con la forma attivata, libera dell'enzima. L'attivazione modificherebbe perciò l'accessibilità del gruppo ferro non eme.

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Effects of Tetrahydrocannabinols on Cyclic AMP Levels in Rat Brain Areas

The role of adenosine 3′5′-monophosphate (cyclic AMP) as an intracellular mediator of hormone action has been well established[1]. Goldberg[2] had previously reported that dibutyryl cyclic AMP potentiated the end plate potential in isolated rat diaphragm by apparently facilitating the release of acetylcholine. *Trans*-(−)-Δ^8-Tetrahydrocannabinol (Δ^8-THC) has been shown to have an effect similar to that of anticholinergic agents in abolishing the behavioral inhibition of habituating experiences in mice[3]. In a previous paper we reported decreases in rat brain acetylcholine levels after injection of Δ^8- and Δ^9-THC[4]. Therefore, we thought that the action of the THC's on the cholinergic system could possibly be mediated by cyclic AMP. This report describes the effect of the THC's on the concentration of rat brain cyclic AMP.

Table I. Effects of Δ^8-THC and Δ^9-THC on cyclic AMP levels in rat brain areas

Brain area	Cyclic AMP		
	Control	Δ^8-THC	Δ^9-THC
Cortex	0.17 ± 0.08	0.29 ± 0.04	0.14 ± 0.09
Hypothalamus	2.39 ± 0.65	2.19 ± 0.33	2.26 ± 0.27
Cerebellum	1.18 ± 0.16	1.08 ± 0.05	1.17 ± 0.21
Medulla	1.25 ± 0.30	1.03 ± 0.08	1.20 ± 0.12
Midbrain	0.84 ± 0.17	1.29 ± 0.17[a]	0.88 ± 0.09

1 h after receiving either 10 mg/kg of the THC or 4% Tween 80-saline i.v. brain areas from rats were assayed for the level of cyclic AMP (pmole/mg wet tissue). Each value represents the mean (± S.E.) of 8 determinations. [a] Significantly different from the vehicle control, $p < 0.05$.

Methods. Male Sprague-Dawley rats in groups of 4 were injected i.v. with 10 mg/kg of Δ^9-THC or Δ^8-THC in 4% Tween-80-saline and sacrificed after 1 h. Animals used in the determination of cyclic AMP levels were sacrificed in a

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Table II. Effect of Δ^8-THC on adenylate cyclase and cyclic nucleotide phosphodiesterase activities in rat brain areas

Brain area	Adenylate cyclase		Cyclic nucleotide phosphodiesterase	
	Control	Δ^8-THC	Control	Δ^8-THC
Medulla	0.11 ± 0.02	0.10 ± 0.10	7.94 ± 0.84	8.67 ± 0.25
Cerebellum	0.58 ± 0.09	0.33 ± 0.08	13.79 ± 1.24	17.06 ± 1.56 [a]
Midbrain	0.93 ± 0.13	0.68 ± 0.07 [b]	70.68 ± 12.79	54.85 ± 7.04 [b]

1 h after receiving either 10 mg/kg of Δ^8-THC or 4% Tween 80-saline i.v., brain areas from rats were assayed for adenylate cyclase (nmole cyclic AMP formed/mg protein/min) and cyclic nucleotide phosphodiesterase (μmole inorganic phosphate released/mg protein/20 min) activities. Each value represents the mean (± S.E.) of 8 determinations. [a] Significantly different from the vehicle control, $p < 0.01$. [b] Significantly different from the vehicle control, $p < 0.02$.

commercial microwave oven by exposure to microwaves for 30 sec[5]. The heads were chilled before removal of brains and dissection into various brain areas; other animals were sacrificed by decapitation. Brain areas were identified according to VALZELLI[6].

Cyclic AMP content was determined by the method of GILMAN[7] involving binding of the cyclic nucleotide to protein kinase. Tissues were homogenized in 5% TCA and extracted with ethyl ether. The aqueous layer was evaporated to dryness under dried nitrogen and the residue containing cyclic AMP was incubated with protein kinase, ^{3}H-cyclic AMP and sodium acetate buffer, pH 4. Samples were calculated by reference to a standard curve.

The method of WEISS and COSTA[8] was used for estimation of adenylate cyclase activity with the labeled cyclic AMP formed from the substrate, ^{14}C-ATP, being purified by column chromatography and precipitation by barium hydroxide and zinc sulfate. Samples were corrected for recovery. Cyclic nucleotide phosphodiesterase was assayed by the method of ROBERTS[9] wherein the 3′, 5′-phosphodiester bond of cyclic AMP was cleaved by the phosphodiesterase, followed by the cleavage of 5′-phosphate by snake venom esterase. Inorganic phosphate content was then determined by the method of CHEN[10].

Results and discussion. While Δ^9-THC exerted no significant changes in cyclic AMP levels in the rat brain areas examined, its isomer, Δ^8-THC, produced a significant increase of cyclic AMP content in the midbrain and slight decreases of the cyclic nucleotide in the cerebellum and medulla (Table I). When the biosynthetic and metabolic enzymes were assayed in the three brain areas, Δ^8- THC effected a significant decrease in the activities of both adenylate cyclase and cyclic nucleotide phosphodiesterase in the midbrain. It is therefore likely that the elevation of cyclic AMP in the midbrain by Δ^8-THC could be the result of a decreased turnover of the cyclic nucleotide.

A number of psychoactive drugs, such as benzodiazepines, phenothiazines, tricyclic antidepressants and purine stimulants have been known to affect brain cyclic AMP systems via the inhibition of phosphodiesterase. A correlation has been found between the ability of these drugs to reduce anxiety and their ability to inhibit phosphodiesterase implying that the drug action may be mediated by brain cyclic AMP[11,12]. In a previous report[4], rat brain acetylcholine was shown to be decreased by both Δ^8-THC and Δ^9-THC. However, it is not likely that the effect of THC's on the cholinergic system is mediated by cyclic AMP, because the present study has shown a lack of effect of the Δ^9-isomer on the nucleotide level in several rat brain areas. The difference observed with the 2 isomeric THC's regarding their effects on the cyclic AMP system further substantiates the report from our group and that of SEGAL and KENNY[13] that the two isomers exert different spectra of biochemical and psychopharmacological actions. The mediation of some behavioral effects of Δ^8-THC by cyclic AMP is highly possible.

Zusammenfassung. Δ^8-Tetrahydrocannabinol senkt die Adenylcyclase und die Phosphodiesterase im Mittelhirn und verursacht gleichzeitig eine Erhöhung des cAMP-Gehaltes.

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Polyhydroxylated Metabolites of *trans*-Stilbene in the Rat

Several stilbene derivatives, most notably the estrogenic compound diethylstilbestrol, are of interest because of their considerable biological actions. However, our knowledge of the metabolism of stilbenes is not extensive, although some recent investigations have provided several interesting findings. Thus, *trans*-stilbene itself, which was earlier shown to be converted to the extent of a few percent to 4,4′-dihydroxystilbene in rabbits[1,2], is now known to undergo a significant degree of hydroxylation in both rabbits and guinea-pigs[3]. The urinary metabolites,