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.

W. E. ASKEW[14] and B. T. Ho

Texas Research Institute of Mental Sciences, 1300 Moursund, Texas Medical Center, Houston (Texas 77025, USA), and Department of Biophysical Sciences, University of Houston, Houston (Texas 77004, USA), 7 February 1974.

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[14] Present address: Department of Psychiatry, Baylor College of Medicine, Houston (Texas 77025, USA).

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,

which amounted to 10–11% of the dose in these species, were identified as 4-hydroxystilbene, 4,4′-dihydroxystilbene and both of the monomethyl ethers of 3,4-dihydroxystilbene. In adition, a novel metabolite, 4,4′-dihydroxybibenzyl, which was formed by the reduction of the double bond was reported to account for about 2% of the administered *trans*-stilbene in rabbits[4]. Further relevant findings about the metabolic fate of stilbenes have been obtained in studies of a dihydroxy derivative, diethylstilbestrol. In the rat, this compound is extensively excreted in the bile as a glucuronide conjugate which is subsequently hydrolyzed by intestinal bacteria, thus giving rise to a pronounced enterohepatic circulation of diethylstilbestrol[5–7]. In view of the above findings, together with the knowledge that reductive reactions are often carried out by the intestinal bacteria[8], the present study was carried out in order to investigate the nature and origin of the hydroxylated metabolites of *trans*-stilbene in the rat.

Male albino rats weighing approx. 250 g were used. They were maintained on a commercial pellet diet and water ad libitum but, in some experiments, they were switched to a purified diet[9] three days prior to dosing. *Trans*-stilbene, a zone-refined sample of more than 99.9% purity, was administered at a dose level of 100 mg/kg intragastrically as either an aqueous suspension or a solution in soya oil or i.p. as a solution in soya oil (1 ml). Urine and faeces samples were collected separately in containers kept at $< 0\,^{\circ}C$. A preliminary study indicated that the urinary excretion of free metabolites of stilbene amounted, at most, to traces only and the urine samples were therefore hydrolyzed prior to extraction. Portions of samples hydrolyzed by refluxing for 1 h in 1 *N* HCl showed reduced amounts of metabolites compared with indentical samples incubated with a glucuronidase-sulphatase preparation (0.1 ml of glusulase, Endo Laboratories, Garden City, N.Y. in 10 ml of sample). Furthermore, maximal amounts of metabolites were detected following an incubation time of approx. 6 h and reduced recoveries were noted when longer times were used. These samples were then extracted with ether which was shaken with 5% $NaHCO_3$ solution to remove acidic com pounds. The ether extracts were dried, evaporated and treated with 1 ml Tri-Sil (Pierce Chemical Co., Rockford, Illinois). The trimethylsilyl ethers of the metabolites thus formed were analyzed by gas chromatography and combined gas chromatography/mass spectrometry (Varian MAT 111) using columns containing 3% OV-17 or OV-25.

The results showed that variations in the route of administration or dosage form of *trans*-stilbene or in the diet given, resulted largely in alterations in the quantitative rather than the qualitative nature of the pattern of urinary metabolites. Urinary excretion of metabolites was more prolonged in animals fed the highly digestible purified diet (up to 10 days) whereas it was essentially complete within 4 days when the ordinary diet was used. As a result of comparisons with the retention times and the mass spectra of reference compounds, the following metabolites were unequivocally detected: 4-hydroxy-, 4,4′-dihydroxy- and 3,4,4′-trihydroxy-stilbene; 3,4-dihydroxy-, 4,4′-dihydroxy-, 3,4,4′-trihydroxy- and 3,3′,4,4′-tetrahydroxybibenzyl. These remarkable tri- and tetra-hydroxylated metabolites were found to be the major urinary metabolites of *trans*-stilbene. An indication of the amounts of hydroxylated metabolites excreted was obtained by adding the chemically related 4-hydroxydiphenylmethane to the urine samples as an internal standard. The recovery after 4 days in rats fed the ordinary diet amounted to about 24% of the dose and the respective values for trihydroxybibenzyl, trihydroxystilbene and tetrahydroxybibenzyl were approximately 8, 2 and 5% of the dose. Furthermore, smaller amounts of the monomethyl ethers of all three of these major metabolites were also identified. In the case of the bibenzyl derivatives, the mass spectra of these metabolites show a prominent peak at *m/e* 209 which is highly characteristic for the TMSO (CH_3O)-benzyl fragment.

The metabolites excreted in the bile collected for 3 or 5 h following the i.p. injection of *trans*-stilbene were found to be 4-hydroxy-, 4,4′-dihydroxy- and 3,4,4′-trihydroxystilbene. Metabolite excretion was greater in the 5 h sample, which also contained relatively more di- and trihydroxy compounds. No bibenzyl compounds were detected in the bile. The above 3 stilbene derivatives, together with the dihydroxy-methoxy compound, were excreted in the urine of rats in which the common bile duct was tied off and severed prior to the stilbene injection. These rats did not excrete bibenzyl metabolites in the urine. Another experiment, in which 2 rats were fed the ordinary diet supplemented with 1% neomycin sulphate beginning 3 days prior to dosing, showed a striking reduction in the amounts of urinary phenolic metabolites. Less than 1% of the dose was found in the 2-day urine and this consisted mainly of 4,4′-dihydroxy- and 3,4,4′-trihydroxystilbene. Some methyl ether of the latter compound was also detected but of the bibenzyls, only a small amount of the 4,4′-dihydroxy derivative was present. These results strongly suggest that reduction of the double bond in the hydroxylated metabolites of *trans*-stilbene is a reaction carried out by the intestinal microflora following the biliary excretion of the stilbenes. The degree of reabsorption of the biliary metabolites and their intestinal products is not known, but the present study, which has accounted for only about $^1/_4$ of the administered *trans*-stilbene, suggests that faecal excretion is also likely. Technical difficulties have not permitted a clear assessment of this point yet but further work is planned with ^{14}C-labelled *trans*-stilbene, which will hopefully provide a more comprehensive picture of the metabolic fate of this compound[10].

Zusammenfassung. Nach Eingabe von *trans*-Stilben bei Ratten wurden die grössten Mengen im Harn als 3,4,4′-Trihydroxystilben, 3,4,4′-Trihydroxybibenzyl und 3,3′,4,4′-Tetrahydroxybibenzyl nachgewiesen. Die Bibenzylderivate werden nachscheinlich nach Ausscheidung in der Galle von Darmbakterien metabolisiert.

R. R. Scheline

Department of Pharmacology, School of Medicine, University of Bergen, MFH-Bygget, N–5000 Bergen (Norway),
21 January 1974.

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[10] The author wishes to thank Mr. E. Solheim for assistance with GC/MS analyses and Mrs. Astrid Hetle and Mrs. Eli Tepstad for technical assistance. Grants from Norges Almenvitenskapelige Forskningsråd and Norsk Medisinaldepot for the purchase of the GC/MS system are gratefully acknowledged.