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DISTRIBUTION OF RADIOACTIVITY IN BRAIN OF TOLERANT AND NONTOLERANT PIGEONS TREATED WITH ^{3}H-Δ^9-TETRAHYDROCANNABINOL*

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Abstract—Levels of radioactivity in the brain stem, cerebellum, temporal cortex and frontal cortex of birds tolerant to Δ^9-tetrahydrocannabinol (Δ^9-THC) did not differ significantly from levels in the same tissue of nontolerant birds after administration of ^{3}H-Δ^9-THC. Levels of radioactivity in the lungs of both tolerant and nontolerant birds were similar to the levels of radioactivity in the brain areas after ^{3}H-Δ^9-THC; however, higher levels of radioactivity were found in the livers of both groups of birds than were found in the brains. Approximately 0·1 per cent of the total dose of radioactivity was in the brain of both tolerant and nontolerant birds 2·5 hr after injection. When pigeons were injected repeatedly (seven times in 2 weeks) with ^{3}H-Δ^9-THC, there was some accumulation of radioactivity in brain and lung, and an even greater accumulation in liver. These data suggest that tolerance to Δ^9-THC in pigeons is not due to a decreased concentration of total cannabinoids in the brain.

WE HAVE reported[1] that levels of Δ^9-tetrahydrocannabinol (Δ^9-THC) and its metabolites in the blood of pigeons tolerant to Δ^9-THC do not differ from the levels in nontolerant birds. On the basis of this evidence, we suggested that tolerance to Δ^9-THC in the pigeon probably is not metabolic.

The importance of measuring brain levels of drugs in the study of tolerance mechanisms has been emphasized by Kalant *et al.*[2] They suggest that for drugs which act on the CNS it is possible for blood levels of drugs to be the same in tolerant and nontolerant animals, while the ratio of brain to blood levels differs. If it can be shown for drugs which affect the CNS that the levels of these drugs are as high as or higher in the brains of tolerant animals than they are in nontolerant animals, it is strong evidence that the tolerance mechanisms are not drug distributional. For example, Mule and Woods[3] have shown that the concentration of morphine in the brains of morphine-tolerant dogs was at least as high as the concentration of morphine in the brains of dogs not tolerant to morphine after both groups of dogs had received the same dose of morphine. These data have often been interpreted to mean that tolerance to morphine occurs within the brain, rather than by a mechanism limiting the amount of drug entering the brain.[4]

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With these considerations in mind, we injected ^{3}H-Δ^9-THC into birds tolerant to Δ^9-THC and into birds that had not received Δ^9-THC previously, and then measured the radioactivity in various brain regions in an attempt to determine if tolerance to Δ^9-THC in pigeons results from a decreased concentration of cannabinoids in the brain of tolerant birds.

METHODS

Adult male White Carneaux pigeons weighing between 400 and 580 g when given free access to food and water were used in these experiments. All birds were deprived of food until they reached 80 per cent of their free-feeding weight and were subsequently maintained at these weights during the experiments. Four pigeons were used per group.

The experimental chambers for pigeons were a modification of the sound-attenuating chambers of the type used by Ferster and Skinner.[5] A translucent plastic response key, 2 cm dia., was mounted on a wall inside each chamber. The minimum force required to operate the key was about 15 g. Opening of the key contacts defined a pecking response. The key was transilluminated by blue lamps. Directly below the key was a rectangular opening through which the pigeon could be given 4-sec access to grain after 30 responses. The chamber was illuminated by a 25-watt bulb, and white noise was present at all times.

The fixed ratio (FR) schedule has been described in detail elsewhere. In these experiments, the thirtieth key peck in the presence of a distinctive key light (blue) produced 4-sec access to grain. An experimental session continued until the bird had obtained 24 food presentations, or until 30 min elapsed, whichever occurred first.

Prior reports from our laboratory have shown that rapid and marked behavioral tolerance develops to Δ^9-THC in pigeons.[6,7] Therefore, after responding under the FR 30 schedule stabilized, four pigeons were injected three times weekly (Monday, Wednesday and Friday) with 10 mg/kg of Δ^9-THC and were tested 2 hr later. On Tuesdays and Thursdays the birds were tested but not injected. Four other birds were given six injections of Triton X-100, the agent used to suspend the insoluble Δ^9-THC for injection, under a similar injection and behavioral testing schedule. The seventh injection given to both groups of birds was 10 mg/kg of Δ^9-THC of which 0·2 mg or 0·2 cc was ^{3}H-Δ^9-THC (specific activity, 24·4 μCi/mg). Another group of four birds was given seven injections of 10 mg/kg of Δ^9-THC of which 0·2 mg was ^{3}H-Δ^9-THC. A final group of four pigeons was injected with 10 mg/kg of Δ^9-THC of which 0·2 mg was ^{3}H-Δ^9-THC. All animals were tested 2 hr after the seventh injection and sacrificed 30 min later by decapitation.

The brain was quickly removed from the skull, and the brain stem, cerebellum, cortex and midbrain were dissected and weighed. A small portion, approximately 20 mg, was removed from each of these regions, except for the cortex where samples of both temporal and frontal cortex were removed. Each sample was weighed and placed on filter paper in petri dishes in the hood to dry. The lungs and liver also were removed and weighed, then approximately a 20-mg portion of each was dissected and prepared as described above. The samples were combusted in a Packard automatic oxidizer after at least 3 days of drying. The scintillant used in these experiments was Permafluor II (Packard Instrument Company, Downers Grove, Ill.), which was added automatically by the oxidizer.

Radioactivity was quantitated in a Beckman LS 100 liquid scintillation counter and correction for quenching was achieved by internal standardization. Negligible quenching was observed with these samples.

The randomization test for two independent samples was used to analyze the data for significant differences,[8] since parametric statistics did not seem appropriate for the group size used in these experiments.

RESULTS

The data in Table 1 show that six injections of Triton X-100 did not affect the key pecking of the four nontolerant birds (group I). However, the intramuscular injec-

TABLE 1. NUMBER OF KEYPECKS BY PIGEONS FOR FOOD UNDER AN FR 30 SCHEDULE BEFORE, DURING AND AFTER CHRONIC ADMINISTRATION OF Δ^9-THC

Group I		Group II		Group III	
Treatment	Total responses	Treatment	Total responses	Treatment	Total responses
No injection	714*	No injection	722†	No injection	717
No injection	711	No injection	723‡	No injection	726
No injection	711	No injection	728	No injection	734
Triton X-100	729	No injection	725	No injection	718
No injection	722	Δ^9-THC	0	No injection	716
Triton X-100	723	No injection	182	^{3}H-Δ^9-THC	0
No injection	730	Δ^9-THC	180	No injection	0
Triton X-100	726	Δ^9-THC	361	^{3}H-Δ^9-THC	178
Triton X-100	741†	No injection	722	^{3}H-Δ^9-THC	1
No injection	731	Δ^9-THC	723†	No injection	665
Triton X-100	732	No injection	722	^{3}H-Δ^9-THC	541
No injection	610†	Δ^9-THC	726	No injection	617
Triton X-100	730	Δ^9-THC	722	^{3}H-Δ^9-THC	384
No injection	728	No injection	721	^{3}H-Δ^9-THC	570
^{3}H-Δ^9-THC	0	^{3}H-Δ^9-THC	557	No injection	645
				^{3}H-Δ^9-THC	400

* Average number of responses for four pigeons.
† Average number of responses for two pigeons.
‡ Average number of responses for three pigeons.

tion of 10 mg/kg of Δ^9-THC completely eliminated key pecking of these birds. The birds (group II) that were given six injections of non-labeled Δ^9-THC prior to the labeled injection also did not respond after the initial injection of Δ^9-THC; however, they responded at or near their pre-drug control rates after their sixth injection, which suggests that these animals were tolerant to the effects of 10 mg/kg of Δ^9-THC. Similar tolerance to the behavioral effects of Δ^9-THC was also observed in the pigeons who were given six injections of labeled THC (group III). The seventh injection for all birds was ^{3}H-Δ^9-THC. The tolerant birds continued to key peck after the dose of ^{3}H-Δ^9-THC, but the birds that had been receiving Triton X-100 injections did not respond at all.

The distribution of radioactivity in the various sections of the brain and in lung and liver of pigeons given ^{3}H-Δ^9-THC is presented in Table 2. Although there was slightly less radioactivity in the brains of the tolerant birds than in the brains of

TABLE 2. RADIOACTIVITY IN TISSUE OF TOLERANT AND NONTOLERANT PIGEONS AFTER 2·5 μCi ^{3}H-Δ^9-THC

Tissue	Nontolerant (dis./min/mg)*	Tolerant (dis./min/mg)*	Tolerant/Nontolerant (dis./min/mg)*
Brain stem	3·3	2·7	0·8
Cerebellum	3·4	2·5	0·7
Frontal cortex	2·4	2·1	0·9
Temporal cortex	2·8	2·3	0·8
Midbrain	3·1	2·2	0·7†
Lung	1·9	2·8	1·5
Liver	14·4	15·8	1·1

* Average of four birds.
† $P < 0.05$ as determined by the randomization test for two independent samples.[8]

the nontolerant birds, this difference was significant only for the midbrain region. In most brain areas there was no evidence of differences in the levels of radioactivity in tolerant and nontolerant birds. Nor were there any significant differences in levels of radioactivity in the lungs and livers of tolerant and nontolerant birds.

The amount of radioactivity per milligram of tissue seemed to be about the same in tissue from lung, brain stem, cerebellum, frontal cortex, temporal cortex and midbrain. However, about five times as much radioactivity was present per milligram of liver tissue as in the other tissues.

The per cent of the total dose of radioactivity administered, recovered from brain, liver and lung at 2·5 hr after the injection and from plasma 2 hr after the injection is presented in Table 3. There was no difference between the per cent of the radioactivity in any of these organs in tolerant vs. nontolerant pigeons.

TABLE 3. DISTRIBUTION OF RADIOACTIVITY IN TOLERANT AND NONTOLERANT PIGEONS 2·5 hr AFTER AN i.m. INJECTION OF ^{3}H-Δ^9-THC

Behavior	Organ	Organ (% total body wt)	Injected ^{3}H in organ (%)
Tolerant	Brain	0·57*	0·10*
Nontolerant		0·54	0·12
Tolerant	Lung	1·2	0·24
Nontolerant		1·2	0·17
Tolerant	Liver	1·8	1·74
Nontolerant		1·4	1·50
Tolerant	Plasma†	4·4 ‡	1·02
Nontolerant		4·4	1·12

* Average of four birds.
† Blood taken 2 hr after the injection.
‡ The per cent total body weight of plasma (specific gravity = 1·1 g/cc) was based on 44 ml plasma/kg of pigeon body weight as reported in *Biology Data Book*, p. 265, Federation of American Societies for Experimental Biology, Washington, D.C. (1964).

The data presented in Table 4 clearly show that radioactivity accumulated in the brains of animals given seven injections of radiolabeled Δ^9-THC, even though those

TABLE 4. RADIOACTIVITY IN TISSUE OF PIGEONS AFTER ONE OR SEVEN INJECTIONS OF 5·0 μCi ^{3}H-Δ^9-THC

Tissue	One injection (dis./min/mg)	Seven injections (dis./min/mg)	Seven injections/One injection
Brain stem	5·3*	11·7	2·2†
Cerebellum	5·4	8·0	1·5†
Frontal cortex	4·6	7·2	1·6†
Temporal cortex	5·5	8·3	1·5†
Midbrain	5·6	8·3	1·5†
Lung	6·5	14·4	2·2†
Liver	22·0	92·9	4·2†

* Average of four birds.
† $P < 0.05$ as determined by the randomization test for two independent samples.[8]

injections were given 2–3 days apart. These findings indicate that after the chronic administration of Δ^9-THC the total levels of cannabinoids in the brains of tolerant animals are actually higher than the levels in the brains of nontolerant birds that have received the same dose of ^{3}H-Δ^9-THC. Despite the fact that the tolerant birds have higher cannabinoid levels in brain after seven doses of ^{3}H-Δ^9-THC than do nontolerant birds after a single ^{3}H-Δ^9-THC injection, the tolerant birds did key peck for food 2 hr after the injection, but the nontolerant birds did not.

The accumulation of radioactivity seemed to be about the same in the various brain areas and in the lung; however, there appeared to be a greater accumulation of radioactivity in the liver than in these other tissues when ^{3}H-Δ^9-THC was administered repeatedly.

DISCUSSION

We have shown that the level of radioactivity in several brain areas of pigeons tolerant to Δ^9-THC does not differ significantly from the level of radioactivity in the same brain areas of nontolerant pigeons after injection of the same dose of tritium-labeled Δ^9-THC. Further, when ^{3}H-Δ^9-THC is administered repeatedly to pigeons, there is an accumulation of radioactivity in the brain. These data indicate that the total levels of cannabinoids in the brains of tolerant pigeons are at least as high as, and probably much higher than are the total levels of cannabinoids in the brains of nontolerant birds. On the basis of these data, it does not appear likely that tolerance to Δ^9-THC develops because repeated administration of Δ^9-THC somehow result in a diminished entry of cannabinoids into the brain. This suggests that the tolerance is not due to an alteration in protein binding of Δ^9-THC in the blood-stream, or a decrease in the ability of cannabinoids to cross the blood–brain barrier.

Although levels of radioactivity in the brains of tolerant animals after a single injection of ^{3}H-Δ^9-THC do not differ from levels of radioactivity in the brains of nontolerant animals, we do not know whether the composition of the cannabinoids is the same in the brains of tolerant and nontolerant birds, since we have not developed methods for extracting the metabolites of Δ^9-THC from tissue. However, since the total levels of radioactivity were the same in the brains of these tolerant and nontolerant pigeons, and since in a previous report we did not find any evidence that

plasma levels of Δ^9-THC or its metabolites differed in tolerant and nontolerant birds after an injection of ^{3}H-Δ^9-THC, it seems unlikely that the composition of the cannabinoids in the brains of tolerant and nontolerant birds differs. When ^{3}H-Δ^9-THC was administered repeatedly, radioactivity did accumulate in both blood[1] and brain (Table 4). The accumulation of radioactivity in blood resulted from increasing levels of the metabolites not soluble in petroleum ether or diethyl ether.

We[9] and others[10] have reported that there is considerable metabolism of Δ^9-THC in the liver and that it is secreted in the bile. As might be expected, in the pigeons significantly greater quantities of radioactivity were observed in the liver than in the other tissues. Further, when ^{3}H-Δ^9-THC was injected repeatedly, radioactivity accumulated in the liver to a greater extent than in the other tissues. Since the 4·2-fold accumulation of radioactivity we found in the liver resembles the 3-fold accumulation we found in blood, and since the accumulated radioactivity in blood was accounted for largely by metabolites of Δ^9-THC, it seems likely that the accumulation of radioactivity in the liver also will prove to be associated with metabolites of Δ^9-THC. Significant amounts of these metabolites may be recirculated in the enterohepatic circulation.[9]

As shown in Table 3, approximately 3 per cent of the total quantity of radioactivity injected into the pigeons was recovered from the brain, lung, liver and plasma of both tolerant and nontolerant pigeons 2·5 hr after injection. Although this quantity seems small, it is in agreement with the data reported by Klausner and Dingell,[10] who administered Δ^9-THC intravenously to rats. We have reported that 26 per cent of the dose of radioactivity administered intravenously to rats is secreted in bile during the first 3 hr after medication.[9] Considerable quantities of the radioactivity also may be in the gastrointestinal tract of the pigeons 2·5 hr after the injection of ^{3}H-Δ^9-THC.

We attempted to quantify the amount of radioactivity that remained at the injection site in a number of pigeons. These results varied greatly, possibly because we did not have a marker in the breast muscle to indicate the depth of the needle. We took small samples of tissue from the breast muscle (10–20 mg) and oxidized these samples as described previously. In one of the samples there was ten times as much radioactivity per milligram than in tissue taken from any other organ. These data suggest that all of the radioactivity had not been absorbed within 2·5 hr after the injection of ^{3}H-Δ^9-THC. However, as pointed out in our previous paper,[1] blood levels of radioactivity when ^{3}H-Δ^9-THC is given by this route of administration do not differ in tolerant and nontolerant pigeons.

In our experiments, the quantity of radioactivity in the lung was similar to that in brain, but less than that in the liver. High levels of radioactivity have been found in lung at short time periods after an intravenous administration of radiolabeled Δ^9-THC in rodents.[11] However, 3 hr after the injection, the level of radioactivity in the lung, as shown by autoradiography, is quite low. Perhaps the distribution of Δ^9-THC and its metabolites in lung is similar to that in brain or perhaps, as previously suggested,[12] the lung metabolizes Δ^9-THC in a manner different from the liver.

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