Pharmacology Biochemistry & Behavior, Vol. 14, pp. 863–869, 1981. Printed in the U.S.A.

Uptake of [^{3}H]Colchicine into Brain and Liver of Mouse, Rat, and Chick[1]

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Received 19 July 1980

BENNETT, E. L., M. H. ALBERTI AND J. F. FLOOD. *Uptake of [^{3}H]colchicine into brain and liver of mouse, rat, and chick.* PHARMAC. BIOCHEM. BEHAV. 14(6) 863–869, 1981.—The uptake of [ring A-4-^{3}H] colchicine and [ring C-methoxy-^{3}H]colchicine has been compared in mice from 1 to 24 hr after administration. Less radioactivity was found in brain after administration of ring-labeled colchicine than after administration of the methoxy-labeled colchicine. Three hr after administration of ring-labeled colchicine, 5% of the label was in liver and about 0.01% of the label was present in brain. Forty percent of the brain radioactivity was bound to tubulin as determined by vinblastine precipitation. After 3 hr, an average of 8% of the radioactivity from methoxy-labeled colchicine was found in the liver and 0.16% in brain. However, less than 5% of the activity in brain was precipitated by vinblastine, and the colchicine equivalent was comparable to that found after administration of the ring-labeled colchicine. The amount of colchicine entering mouse brain after subcutaneous injection is comparable to the minimum behaviorally effective dose when administered to the caudate. The metabolism of [ring C-methoxy-^{3}H] and [ring A-^{3}H]colchicine was also studied in rats. The general pattern was similar to mice; less radioactivity was found in brain after administration of the ring-labeled alkaloid than after administration of methoxy-labeled colchicine. Again, 40–50% of ring-labeled colchicine was precipitated by vinblastine. A much smaller percentage of the methoxy-labeled drug was precipitated by vinblastine than of the ring A-labeled colchicine. These experiments, together with behavioral experiments [7], support the hypotheses that structural alterations in synapses by recently synthesized proteins which are transported down the axons and dendrites may be an essential process for long-term memory formation.

Colchicine Tubulin Mice Rat Chick [Ring-labeled]colchicine [Methoxy-labeled]colchicine Colchicine, uptake in brain Colchicine, uptake in blood Colchicine and memory Axonal transport and memory

THE INITIAL purpose of the biochemical experiments reported here was to estimate the amount of colchicine entering the mouse brain after its subcutaneous administration under the conditions of the behavioral experiments described by Flood *et al.* [7]. During the course of these experiments, it became apparent that significantly less radioactivity entered the mouse brain than had been anticipated based upon the reports of Stewart and Rose [21] from experiments using rats. Subsequent experiments were then performed in rats, mice, and chicks to compare the effects of (a) label position and (b) species upon apparent uptake of colchicine. In addition, the tubulin content of mouse brain was determined by colchicine binding assays, and the ratio of colchicine uptake to tubulin was estimated. While only a small proportion of colchicine enters the brain of mice or rats, the amounts are comparable with the dose shown to be behaviorally effective [7].

METHOD

Animals and Reagents

Swiss-Webster male mice from Charles River Breeding Laboratories, Wilmington, MA were obtained at 6 weeks of age and maintained in our colony until 50–70 days of age, at which time they weighed 30–35 g. The Wag-rij male rats in Experiments 1 and 2 were obtained from the Lawrence Berkeley Laboratory colony and were approximately 70 to 90 days of age. For Experiments 13 and 17, Wistar (Lewis) rats

[1]Presented at the Tenth Annual Meeting of the Society for Neuroscience, Cincinnati, OH, November 1980.
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obtained from Charles River Breeding Lab were used. Animals were maintained on a 12 hr light-12 hr dark cycle and were housed singly for 2–3 days prior to the experiment. Fertilized White Leghorn chicken eggs were obtained from a local hatchery, and the chicks, hatched in the laboratory, were maintained in the incubator room at 37°C until sacrificed.

[Ring A-4-^{3}H]colchicine (in ethanol) was obtained from Amersham Corp. The specific activity of the three batches used in these experiments was 7.7 Ci/mmole. The colchicine was stored in the dark at −10°C and appeared to be stable during the course of the experiments. [Ring C-methoxy-^{3}H]colchicine, (in 9:1 benzene-ethanol) was obtained from New England Nuclear (NEN) and Amersham (Am). The specific activities of the lots were 10.1 and 19.6 Ci/mmole (NEN) and 5.0 Ci/mmole (Am). Immediately prior to use, an appropriate aliquot of the radioactive colchicine solution was evaporated to dryness by a N_2 stream, and redissolved in sterile 0.9% NaCl. The radioactive colchicine was not diluted for the *in vivo* experiments described in this report. For *in vitro* studies to estimate the amount of tubulin in mouse brain, non-radioactive colchicine was added to obtain a final specific activity of approximately 0.04 Ci/mM.

Vinblastine sulfate was obtained from Sigma Chemical Co., St. Louis, MO and Sephadex G-100 from Pharmacia, Piscataway, NJ. Anisomycin (ANI) was purchased from Pfizer Diagnostics, Clifton, NJ; cycloheximide was obtained from Calbiochem-Behring Corp., La Jolla, CA. Colchicine (0.5 mg/ml) was obtained in sealed ampoules from Eli Lilly and Co., Indianapolis, IN. The rpi counting cocktail 3a70B was obtained from Research Products International Corp., Elk Grove village, IL. Woelm silica gel GF thin layer chromatography plates were obtained from ANALTECH, Inc., Newark, DE Other chemicals, reagent grade, were obtained from usual suppliers.

Experimental Procedure

To conform to the drug design used in the behavioral studies [7], anisomycin (ANI) was administered subcutaneously to mice at a dosage of 20 mg/kg in Experiments 3–10. Seventy-five min later, radioactive colchicine was administered subcutaneously in 0.3 ml of 0.9% NaCl. In subsequent experiments, ANI was not administered; results were unchanged. The mice were anesthesized by ether; a blood sample was taken, and then the animal was perfused with normal saline prior to dissection of liver and brain. These two organs were weighed. Typically, two brains (0.4–0.5 g each) were combined and homogenized in 1.0 ml of PMG buffer (10 mM sodium phosphate with 0.1 mM GTP and 5 mM $MgCl_2$, pH 6.8) [18]. The homogenate was centrifuged for 30 min at 20,000 RPM (50,000×G) at 4°C in a Sorvall RC2B centrifuge, SM-24 head. The precipitate was resuspended in 1.0 ml PMG buffer, recentrifuged, and the supernatants combined (S_1). The combined volume of S_1 was typically 1.6–1.8 ml and it contained approximately 25% of the brain protein. Radioactivity was determined on two 200 μl aliquots of S_1. The residual brain was homogenized in 9 ml of H_2O and 0.6 to 1.2 ml aliquots were counted.

To determine the vinblastine precipitable radioactivity, duplicate 200 μl aliquots of S_1 were incubated in a shaking water bath at 37°C with 0.5 mg vinblastine (final concentration 2.5×10^{-3} M) for 30 min and then centrifuged at 50,000×G for 30 min at 25°C [18]. Radioactivity was determined in both the supernatant and the vinblastine precipitate of tubulin which was rinsed with 200 μl of H_2O (40°). To determine the trichloroacetic acid (TCA) precipitable radioactivity, 200 μl cold 12% TCA was added to an equal volume of S_1, and kept in ice for at least 30 min. The precipitate was sedimented in a clinical centrifuge, resuspended in 600 μl cold 6% TCA, and recentrifuged. Radioactivity was determined in supernatant, wash, and precipitate.

To confirm the presence of colchicine in the vinblastine precipitate, the washed vinblastine precipitate was extracted twice with 125 μl of CH_3OH. A known aliquot was counted, and the remainder was spotted onto a silica gel chromatography plate with added carrier colchicine. Two dimensional chromatography was carried out using chloroform/acetone/diethylamine (5:4:1) in the first direction and triethanolamine: dichloromethane: methanol (10:1:1) in the second, The colchicine was located with UV light, eluted, and the radioactivity determined.

The biochemical procedures used for the rat experiments were similar to those used for mice, except that one rat was used for each experiment, no ANI was given, and colchicine was injected intraperitonally rather than subcutaneously to follow the procedure of Stewart and Rose [21]. The volumes used to process a rat brain weighing approximately 2.0 g were twice those used for the mice.

Chicks were administered 22 nmoles of [ring-A-4-^{3}H]colchicine in 50 μl of saline in the heart region following the general description of Cronly-Dillon *et al.* [4]. The chicks were sacrificed 1 or 3 hr after administration of colchicine, and individual brains (800–900 mg) were analyzed by the same biochemical procedure as described for mouse brains.

Livers were homogenized at a concentration of 100 mg/ml in H_2O; 100 μl aliquots were counted in a gelled scintillator solution without further processing. Blood samples (10 μl) were also directly counted.

Tubulin Content of Brain

The tubulin content of mouse brain was determined by gel filtration following described procedures [18,23]. The 50,000×G S_1 supernatant was prepared as described above using homogenate concentrations ranging from 0.05 g brain/ml to 0.5 g/ml. The supernatant was incubated at 37°C for 60 min with 50×10^{-6} M colchicine, sp. act. 88,000dpm/nmole. One ml of this incubation mixture was chromatographed thru a Sephadex G100 column, 1×9 cm, using 10 mM sodium phosphate-10mM $MgCl_2$ buffer, pH 6.8. One ml aliquots were collected. Total radioactivity, vinblastine-precipitable radioactivity, and total protein (determined by the Lowry procedure with bovine serum albumin as standard), were determined on aliquots.

Determination of Radioactivity

Radioactivity was determined with a Packard Model 3385 scintillation counter using mini-vials. Counting efficiency, typically 30–40%, was determined from the AES ratio and checked by internal standards. The counting mixture normally contained 100-500 μl of sample, 1.2 ml of H_2O, and 3 ml of rpi complete counting cocktail 3a70B.

In each experiment, the corresponding tissue from a pair of mice, a rat, or a chick which had not been injected with radioactive colchicine was fractionated by the described procedure, and appropriate aliquots were used for background corrections. This was particularly important for brain samples, especially those from rat, since total counts were

TABLE 1

RADIOACTIVITY IN BLOOD, LIVER AND BRAIN OF MICE AFTER SC ADMINISTRATION OF [Ring A-4-^{3}H] COLCHICINE

	nmoles Adm	% in Blood*	% in Liver	% in Brain	% of Brain Supernatant TCA Insoluble	% of Brain Supernatant Vinblastine Precipitable	Colchicine Equivalent in Brain† (pmoles)
				1 Hr Incorporation Time (2 experiments)			
Average	3.8	0.94%	6%	0.008%	3%	43%	0.14
Range	(3.6–4.0)	0.68–1.2)	(5–7)	(0.007–0.009)	(2–4)	(42–44)	(0.12–0.16)
				3 Hr Incorporation Time (7 experiments)			
Average	4.1	0.26%	5%	0.009%	3%	40%	0.14
Range	(3.5–5.4)	(0.12–0.53)	(2–9)	(0.007–0.011)	(1–4)	(26–54)	(0.10–0.19)
				24 Hr Incorporation Time (1 experiment)			
	4.0	0.22%	0.3%	0.012%	1%	10%	0.05

*The blood volume was assumed to be 78 ml/kg mouse [24]; body weights were 34 ± 3 g, and livers averaged 2 g.
†The colchicine equivalent was calculated from percentage of brain supernatant radioactivity precipitated by 2.5×10^{-3} M vinblastine multiplied by total radioactivity in brain. The result was multiplied by 4 and divided by the actual nmoles injected to normalize to 4 nmole dose, the amount used in the behavioral experiments of Flood *et al.* [7].

low and the blank correction was a significant fraction of the total counts.

RESULTS

The main objective of the first series of experiments was to determine the amount of colchicine entering mouse brain under the conditions of our behavioral experiments. A dose of about 4 nmoles/mouse was chosen because this approximated the behaviorally effective dose. Mice were sacrificed 3 hr after administration of ring-[^{3}H]-labeled colchicine since the behavioral experiments suggested that this period was critical for determining long-term memory formation in these experiments. The mice were administered ANI, but other experiments indicated that ANI had little effect on the uptake of ring-labeled colchicine.

The results, summarized in Table 1, showed that while substantial quantities of radioactivity were present in liver, and moderate quantities in blood, less than 0.01% of the administered radioactivity (0.4 picomoles equivalent colchicine) was found in brain. About 60% of this radioactivity was found in the S_1 supernatant, and 40% remained in the once-washed precipitate.

Approximately 40% of the radioactivity, equivalent to 0.14 pmoles of colchicine, was precipitated with tubulin by 2.5×10^{-3} M vinblastine. A minimum of two-thirds of the radioactivity in the vinblastine precipitate cochromatographed two-dimensionally with carrier colchicine. An approximately equivalent amount of radioactivity (40–50%) was found in samples of brain supernatant dried in an N_2-stream. Little or no radioactivity was precipitated by 6% trichloracetic acid. (Note that 4% of the brain supernatant activity is equivalent to about 200 dpm after administration of 30 μCi of colchicine). Little radioactivity is lost from brain between 3 and 24 hrs after administration of labeled colchicine (Tables 1 and 2).

In a previous report [21] much larger amounts of colchicine were found in rat brain after its administration. The authors did not, however, indicate the labeling position of the colchicine, although, S. P. R. Rose affirmed that methoxy-labeled colchicine was used in their studies (personal communication). Therefore, we designed subsequent experiments to resolve the discrepancies between that report and our results. In the first of these experiments [ring C-methoxy-[^{3}H]colchicine was used instead of ring A-labeled colchicine. Using [ring C-methoxy-^{3}H]colchicine, the proportions of the injected radioactivity retained in blood, liver, and brain of mice were all much higher than had been found with ring A-labeled colchicine (Table 2). However, using methoxy-labeled colchicine the proportion (5%) of the radioactivity precipitated with the tubulin by vinblastine was approximately one-eighth that precipitated by vinblastine after administration of ring A-labeled colchicine. A similar small fraction of the radioactivity remained in N_2-dried samples of the brain supernatant. The best estimate of colchicine equivalent in mouse brain (normalized to a 4.0 nmole dose) was approximately 0.30 pmoles in these experiments with methoxy-labeled colchicine. This is in good agreement with the estimate of 0.14 pmoles obtained from the first series of experiments. In addition, Experiment 10 M, where the dosage was increased by a factor of 8 to 12, showed that the uptake in brain was not saturated over this dose range, but was, instead, proportional to dose. Again, in contrast to the report of Stewart and Rose [21], essentially none of the radioactivity was precipitable by TCA at 0°C.

The next series of experiments compared the uptake of both ring and methoxy-labeled colchicine in the rat at several time intervals (Table 3). Three hr after administration, an even smaller proportion of the injected radioactivity was found in rat brain than had been found with mice. Little, if any, of the radioactivity was precipitable by trichloroacetic acid. As with the mouse, more radioactivity was found in brain after the administration of methoxy-labeled colchicine

TABLE 2

RADIOACTIVITY IN BLOOD, LIVER AND BRAIN OF MICE AFTER SC ADMINISTRATION OF [Ring C-methoxy-^{3}H] COLCHICINE

Exp. No.	nmoles Adm	% in Blood*	% in Liver	% in Brain	% of Brain Supernatant TCA Insoluble	% of Brain Supernatant Vinblastine Precipitable	Colchicine Equivalent in Brain† (pmoles)
				1 Hr			
15 Am-M‡	4.0	2.1	10	0.071	—	5	0.14
14 NEN-M	12.0	1.8	10	0.045	1	9	0.16
Average 1 hr		1.9	10	0.058	—	7	0.15
				3 Hr			
16 NEN-M	0.8	0.8	6	0.12	2	5	0.26
19 NEN-M	1.0	—	—	0.16	2§	—	—
20 NEN-M	1.0	—	—	0.05	4§	—	—
9 NEN-M	1.5	1.6	8	0.17	1	5	0.34
16 Am-M	2.9	0.6	5	0.08	1	6	0.20
15 Am-M	4.0	1.0	4	0.09	1	6	0.20
8 NEN-M	5.8	2.3	13	0.20	1	3	0.24
10 NEM-M	48.0	2.6	12	0.29	1	5	0.58
Average 3 hr		1.5	8	0.16	1	5	0.30
				24 Hr			
16 NEM-M	0.8	0.6	2	0.10	2	10	0.42
16 Am-M	2.9	0.4	2	0.10	2	10	0.42
15 Am-M	4.0	1.0	0.7	0.09	1	4	0.15
Average 24 hr		0.7	2	0.10	1	8	0.33

*See Table 1.

†See Table 1.

‡NEN indicates colchicine obtained from New England Nuclear Corp.; Am signifies Amersham was the supplier.

§In these experiments, our procedure was slightly modified to follow as closely as possible that described by Stewart and Rose [21]. The perfused brain was homogenized in 10 vol of 0.9% NaCl. An aliquot of the supernatant was precipitated by the addition of 2 vol of 10% TCA. The precipitate was washed twice with 10% TCA and counted in RPI-H_2O. A duplicate sample was counted after combustion in a Packard oxidizer. Excellent agreement was obtained between the two methods used to determine radioactivity.

than after the administration of ring-labeled material. Again, a smaller proportion of the methoxy-labeled drug was precipitated by vinblastine in the tubulin fraction than when ring A-labeled material was used. In rat, the amount of vinblastine-precipitable colchicine found in brain at any time was so small as to preclude a determination of the time dependence of uptake.

For a third group of experiments (Expts. 19 and 20, Table 2 and Expt. 21, Table 3), the procedure was modified to conform more closely to that described by Stewart and Rose [21] by (a) reducing the dose of colchicine, and (b) determining the TCA precipitable acitivty in the total brain homogenate prepared in 0.9% saline. The proportion of radioactivity found in the brain was similar to that found in our other experiments, and less than 10% was precipitated by TCA.

The tubulin content of mouse brain was estimated to be a minimum of 3.4 mg/gm (uncorrected for decay) based on the bound radioactive colchicine precipitable after chromatography on a Sephadex column. This is equivalent to approximately 15 nmoles of tubulin/mouse brain or approximately 3% of the total brain protein. This concentration is somewhat less than that reported by Sherline *et al.* [19] for rat brain, but is approximately twice that reported for rat occipital cortex [10].

Inasmuch as Cherfas and Bateson [3] have reported behavioral effects of colchicine in young chicks, a third series

TABLE 3

RADIOACTIVITY IN BLOOD, LIVER AND BRAIN OF RATS AFTER IP ADMINISTRATION OF COLCHICINE

Exp. No.	nmole Adm	% in Blood*	% in Liver	% in Brain	% of Brain Supernatant TCA Insoluble	% of Brain Supernatant Vinblastine Precipitable	Colchicine Equivalent in Brain† (pmoles)
				[Ring A-4-^{3}H]-Colchicine			
				1 Hr			
13R	14.8	0.36	0.8	0.0018	5	52	0.15
17R	16.4	0.50	8.5	0.0020	7	56	0.18
				3Hr			
1R	1.1	0.22	2.1	<0.01	—	—	—
17R	16.4	0.23	2.6	0.0024	4	40	0.15
12R	17.8	0.16	5.4	0.0062%	4	4	0.04
				24 Hr			
13R	14.8	0.13	0.08	0.0030	1	3	<0.02
17R	16.4	0.13	0.02	0.0006	1	<10	<0.02
				[Ring C-methoxy-^{3}H]Colchicine			
				1 Hr			
13 Am-M	16.8	[0.07]	0.4	0.0017	0	11	0.03
17 Am-M	12.1	0.30	0.4	0.0096	1	8	0.12
				3 Hr			
21 NEN-M	2.0	—	—	0.014	10‡	—	—
13 Am-M	16.8	0.18	1.1	0.0020	5	18	0.06
12 NEN-M	15.5	0.30	5.0	0.0236	3	5	0.20
17 Am-M	12.1	0.33	1.0	0.0071	4	12	0.14
				24 Hr			
17 Am-M	12.1	0.47	0.5	0.0128	3	7	0.14
13 Am-M	16.8	0.20	0.1	0.0052	1	3	0.02

*The blood vol was assumed to be 58 ml/kg rat [8]. For Exp. 1, the Wag-rij rat weighed 207 g, the liver weighed 8 g. For Exp. 12, rats weighed 370 g and had 22 g livers. In Exp. 13, Wistar rats weighed 290 g, livers 15 g. The rats used in Exp. 17 weighed 220 g, livers 9 g, and in Exp. 21, the rat weighed 130 g.

†The colchicine equivalent was calculated from the percentage of brain supernatant radioactivity precipitated by 2.5×10^{-3} M vinblastine multiplied by the total radioactivity in the brain. The result was multiplied by 16 and divided by the actual dose administered to normalize to a 16 nmole dose, the approximate average of the dose used in the majority of these experiments. In this experiment, the cortex and subcortex of the perfused brain were each homogenized in 10 vol of 0.9% saline.

‡The radioactivity was determined in the homogenates by the same procedure as used in Exps. 19 and 20, Table 2.

of experiments was carried out to provide data concerning colchicine uptake into the young chick brain. In spite of administration near the heart and the young age of the chick, the amount present in brain (Table 4) was similar to that present in the mouse.

DISCUSSION

On the basis of a limited number of studies, it is generally accepted that the blood-brain barrier completely excludes colchicine from the central nervous system. In an early study, Back *et al.* [1] used [^{14}C]colchicine and concluded that "labeled colchicine was not present in blood, brain, muscle, and heart" of mice. However, the limitation of the low specific activity (27 μCi/g) of the colchicine should be borne in mind in evaluating their results. Subsequently, Hunter and Klaassen [9] studied the metabolism of [^{3}H]colchicine in a variety of species including rat. The majority of the administered colchicine was excreted in the bile, and a lesser amount was excreted in the urine. The investigators reported that the concentration of tritium in brain was the lowest of all tissues sampled, and was 1% of that of the liver 20 min after

TABLE 4

RADIOACTIVITY IN BLOOD AND BRAIN OF CHICKS AFTER IP ADMINISTRATION OF COLCHICINE*

Age of chick	Duration of colchicine administration	% injected found in Blood	Brain
20 hr	1 hr	0.9%	0.034%
20 hr	3 hr	0.15%	0.020
	3 hr	not done	0.027
10 hr	3 hr	0.5	0.064
	3 hr	0.3	0.030

*[Ring-A-4-^{3}H]colchicine (22 nmoles) in 50 μl saline was injected into the heart region of chicks. One or three hr later chicks were decapitated. The blood vol was assumed to be 2.0 ml. Sixty–seventy percent of the radioactivity in brain was precipitated by vinblastine or co-chromatographed with tubulin peak on a G-100 Sephadex column. These values were measured on the approximately 50% of the radioactivity in brain that extracted into S_1 by our procedures.

intravenous administration of colchicine. We found that the maximum proportion of [ring A-4-^{3}H]colchicine in mouse brain 3 hr after administration was about 0.01% of that administered. Approximately 40–50% of the radioactive material in brain was vinblastine precipitable, presumably bound to tubulin.

Stewart and Rose [21] found that a minimum of 0.1% of intraperitoneally administered [ring C-methoxy-^{3}H] colchicine was present in rat cortex. They reported that radioactivity in brain was largely precipitated by 10^{-2} m vinblastine or 6% TCA.

Our studies used both ring- and methoxy-labeled colchicine. The uptake of [ring-4-^{3}H]colchicine or [ring C-methoxy-^{3}H]colchicine in rat brain was substantially less than that in the mouse; much less than 0.01% of the administered dose was found in brain either 3 or 24 hr after administration. Thus, when 60 μCi of methoxy-labelled colchicine was injected, less than 20,000 dpm was found in brain. In the experiment using ring-labelled colchicine, 120 μCi of colchicine was administered and typically less than 8,000 dpm was found in the brain homogenate. The unexpectedly small portion of the colchicine which was found in the rat brain precluded the accurate determination of the relationship of uptake to dose. Thus, in experiment 1R (Table 3) in which approximately 1.1 nmoles (8 μC_1) of colchicine was administered, less than 2000 dpm was found in the brain extract, and we were unable to obtain reliable data on the vinblastine precipitate due to the extremely small amount of radioactivity present. In experiment 21 NEM (Table 3) in which 2.0 nmoles (40 μc) of colchicine was administered, only 12,000 dpm was found in the total brain, and 10% of the radioactivity was precipitated by TCA. It thus appears unlikely that the low percentage uptake in our experiments is due to saturation. However, the apparent uptake of colchicine was somewhat influenced by the label position; again, more uptake was found when the methoxy-labeled colchicine was used. Up to 20 times as much radioactivity was found in mouse brain after administration of methoxy-labeled colchicine than after administration of ring-labeled drug. Schönharting *et al.* [17] showed that rat and mouse liver microsomes demethylate colchicine. Hunter and Klassen [9] showed that significant quantities of demethylated derivatives of colchicine are present in bile and urine of rats after administration of [^{14}C]colchicine. Therefore, we suggest that much of the radioactivity in brain after administration of ring-C-methoxy colchicine may be derived from this liberated methyl group and may include ^3H_2O. This suggestion is supported by our observation that the non-volatile radioactivity in the brain homogenate supernatant corresponded closely with vinblastine-precipitable radioactivity in the same fraction.

A significant fraction of the radioactivity in the brain supernatant after administration of ring-labeled colchicine was precipitated by vinblastine, but little or no radioactivity was precipitated by TCA. Furthermore, in our hands, [^{3}H]colchicine bound to tubulin *in vitro* by standard procedures [18] was dissociated from tubulin by TCA, but was precipitated, as expected, by 2.5×10^{-3} M vinblastine.

Knowledge of the maximum amount of colchicine (0.14 pmoles, average concentration, 0.3×10^{-9} M) present in mouse brain after administration of 4 nmoles of colchicine combined with an estimate of the tubulin content of mouse brain (7.5 nmoles) permits an estimate of the average colchicine/tubulin ratio after subcutaneous administration. This ratio is approximately 1:10^5. However, much of the tubulin is present as microtubules in the brain. It is generally believed that colchicine interferes with microtubule formation by first binding to free tubulin. The tubulin-colchicine molecules subsequently are bound to the growing ends of microtubules and impair continued growth. In this way, relatively small concentrations of colchicine, resulting in colchicine-tubulin ratios which are several orders of magnitude less than unity, can impair biological processes. Crothers and McCluer have shown that intracerebrally administered colchicine remains localized near the site of injection [5].

The impairment of axonal transport by colchicine in both peripheral and central pathways has been reported frequently (e.g. [2, 6, 11, 14, 20]). Levin [12] has recently made a detailed comparison of the effects of colchicine, cytochalasin B, and 6-hydroxydopamine on the transport and subcellular distribution of proteins, tyrosine hydroxylase, dopamine-β-hydroxylase, and norepinephrine in the noradrenergic neurons of the rat locus coeruleus. While colchicine did not impair protein synthesis, it caused total or near total blockade of both fast and slow waves of transported protein or glycoprotein but did not affect a wave of intermediate rate. The block of protein transport was long-lasting, persisting for at least 7 days after colchicine injection. Ultrastructural changes were not found in microtubules 7 days after colchicine administration.

Extremely low concentrations of colchicine interfere with numerous other biological processes. Olmsted and Borisy [15] estimated that a colchicine to tubulin binding ratio of 1:25 will completely inhibit polymerization. Owellen *et al.* [16] calculated that colchicine inhibited tubulin polymerization by 50% as measured by viscosity at a molar concentration of 5×10^{-7} and a colchicine-to-tubulin ratio of about 1:250. Margolis *et al.* [13] showed recently that 1.3×10^{-7} M colchicine inhibits microtubule assembly under steady-state conditions *in vitro* by 50%. In these experiments, the colchicine to tubulin ratio was approximately 1 to 50. Vinblastine, an effective amnestic agent in the behavioral experiments of Flood *et al.*, inhibits the assembly-disassembly reaction of tubulin at even lower concentrations [15, 16, 23]. One striking example of a biological action at an extremely low concentration is the demonstration by Taylor [22] that

mitosis of human cells in tissue culture is completely arrested by a colchicine concentration of 5×10^{-8} M. Thus the observed effects of colchicine on memory formation in mice [7] are not ruled out by the low uptake of colchicine into brain as measured in the present series of experiments.

ACKNOWLEDGEMENT

We wish to express our appreciation to Professor Mark R. Rosenzweig for many helpful suggestions during the preparation of this manuscript. This work was supported by the Assistant Secretary of Environment, Office of Life Sciences Research and Nuclear Medicine Applications, Division of General Life Sciences of the U. S. Department of Energy under contract W-7405-ENG-48 and from National Institute of Mental Health Grant No. 26608-02 to Murray E. Jarvik, M.D., Ph.D.

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