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METABOLISM OF [^{14}C]-SEROTONIN IN THE CAUDATE NUCLEUS, HYPOTHALAMUS AND RETICULAR FORMATION OF THE RAT AFTER ETHANOL ADMINISTRATION

M. TYTELL and R. D. MYERS

Laboratory of Neuropsychology, Purdue University, Lafayette, Ind. 47907, U.S.A.

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Abstract—By means of push–pull perfusions of localized areas in the caudate nucleus, lateral hypothalamus or midbrain reticular formation, the metabolism *in vivo* of [^{14}C]-5-hydroxytryptamine ([^{14}C]-5-HT) injected intracranially was examined in rats which drank ethanol chronically, rats given ethanol acutely and in controls. A significantly greater conversion of [^{14}C]-5-HT to [^{14}C]-5-hydroxyindoleacetic acid ([^{14}C]-5-HIAA) was observed in the caudate nucleus of the acute ethanol rats as compared to the controls. In the ethanol drinkers, the mean levels of [^{14}C]-5-HT and [^{14}C]-5-HIAA generally fell between those of the control and acute ethanol rats. No large differences were found between the three groups in the production of [^{14}C]-5-hydroxytryptophol ([^{14}C]-5-HTOL) within the localized areas of perfusion. It appears that in some parts of the central nervous system, the presence of ethanol may result in increased degradation of 5-HT to 5-HIAA, but does not result in a greatly increased production of 5-HTOL, as seen in the periphery. These results obtained with the conscious animal further suggest that an interaction exists between 5-HT metabolism and ethanol consumption and provide support for earlier work indicating that the relationships may be different for peripheral systems and the brain.

AN IMPORTANT interaction may exist between the putative neurotransmitter, serotonin, and the addictive drug, ethanol. Olson *et al.*[1] observed that ethanol could influence serotonin metabolism in humans, since urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA) decreased in the chronic alcoholic. That this alteration was not simply an inhibition of serotonin metabolism was indicated by the discovery of an elevated level of 5-hydroxytryptophol (5-HTOL), the product of reductive serotonin metabolism in the urine after consumption of ethanol.[2,3]

Studies of ethanol's effects on serotonin levels and metabolism in animals have led to conflicting reports. Acute ethanol administration was found by some researchers to increase brain serotonin content,[4–6] to decrease it,[7,8] or to have no effect at all.[9–12] Chronic administration of ethanol was also observed to decrease serotonin levels,[10] but other investigators have reported evidence suggesting that this does not occur.[6,11]

With respect to the rate of turnover of serotonin, ethanol may have an inhibitory effect[6,12] whereas it increases the rate of serotonin synthesis.[13] A shift in the pattern of metabolism from 5-HIAA to 5-HTOL occurs only after the systemic administration of [^{14}C]-5-hydroxytryptophan ([^{14}C]-5-HTP)[14] or after the incubation of liver slices in a medium containing serotonin and ethanol;[15] however, intracerebral administration of labeled serotonin after a dose of ethanol did not result in a change

in the pattern of degradation.[16] The per cent of the total carbon-14 activity constituted by 5-HTOL in urine collected for 24 hr rose above control levels in rats given ethanol,[17] but this increase was extremely modest in comparison to the levels of 5-HTOL detected in the urine of human subjects after consuming ethanol.[2,3]

Since cerebral serotonin has been implicated in the volitional intake of ethanol,[18–20] the present study was undertaken to examine metabolism of [^{14}C]-5-HT in the brain of the rat after the animal had consumed this fluid over a long period of time or following the acute administration of a sedating dose. Three different anatomical loci of the rat brain were studied: the caudate nucleus, lateral hypothalamus, and midbrain reticular formation. The mesencephalic reticular formation is morphologically implicated in the phenomenon of sleep[21] and the lateral hypothalamus in ethanol selection.[22,23] The caudate nucleus seems to play an important role in the control of motor function[24,25] which is impaired as a result of ethanol ingestion. In this study, each of these structures was perfused by means of push–pull cannulae,[26] in order to determine whether serotonin or its metabolites change as a result of prolonged ethanol intake or acute ethanol sedation.

METHOD

Surgical and experimental procedures. According to procedures described previously,[27] thin-wall 20-ga stainless-steel guide tubes, 10 mm in length, were implanted stereotaxically, under aseptic conditions in the brains of nine adult hooded rats of the Royal Victoria strain. After the guides were passed through bur holes in the skull and 0·5 mm below the dura mater, they were affixed permanently with cranioplast cement. The coordinates for the three sites to be perfused in each rat were determined using the stereotaxic atlas of Pellegrino and Cushman,[28] as follows: caudate nucleus, AP = 8·0, L = 3·0, V = 4·0; lateral hypothalamus AP = 5·8, L = 1·8, V = 8·0; midbrain reticular formation, AP = 0·0, L = 1·9, V = 4·0. Each tube was kept sealed by a removable stylet which was the same length as the guide. After surgery, the animals were allowed 48 hr for recovery.

The animals were divided equally into one of three groups: (1) those which drank ethanol chronically; (2) those given ethanol acutely; and (3) controls. The chronic ethanol animals were given a solution which contained 8% (v/v) ethanol and 2% malt extract (Premier Malt Products, Inc., Milwaukee, Wisc.) as their sole drinking fluid. The extract, used to enhance alcohol intake,[19] was diluted with water to a 60% (w/v) solution to simplify the measurement of specific quantities of the malt, and the entire solution was then passed through a fluted filter paper to remove the suspended solids. The other two groups of rats had plain tap water continuously available. Powdered Wayne Lab Blox was given to all of the animals *ad lib.*; food and fluid intakes were recorded daily and body weight was measured every other day.

After 130–147 days had elapsed, each of the three anatomical sites was labeled with a micro-injection of [^{14}C]-5-HT and subsequently perfused with a physiological salt solution using the push–pull technique.[26] The chronic ethanol and control animals were unrestrained during the micro-injection and perfusion procedures. On the other hand, the acute ethanol rats were sedated after the administration of 6 g/kg of ethanol in a 20% (v/v) solution given by an intragastric tube 30 min before the micro-injection of the [^{14}C]-5-HT.

To examine the magnitude of the effect of ethanol sedation, one of the control animals was also injected and perfused under the same conditions as the acute ethanol group of rats, with 14 days separating the two experiments. As an additional control, the acute ethanol portion of this study was repeated with an interval of 26 days between the initial experiment and the replication.

Micro-injection [^{14}*C*]-5-*HT*. The micro-injection cannula was fashioned from 27-ga stainless-steel tubing, beveled at the injection end, according to procedures described previously.[27] The exact depth of the injection, determined by length of a polyethylene collar, was either 4·0 or 8·0 mm.

The cannula and PE-20 tubing attached to it were filled with a salt solution which contained 127·65 mM Na^+, 2·55 mM K^+, 1·26 mM Ca^{2+}, 0·93 mM Mg^{2+} and 134·58 mM Cl^-.[29] A small air bubble was injected into the PE tubing so that the flow during the injection could be continuously monitored. [^{14}C]-5-HT creatinine sulfate with a sp. act. of 58 mCi/m-mole (Amersham/Searle Corp.) was added to 180 μl of 0·01 N HCl, yielding a stock solution containing 0·278 μCi/μl which was kept refrigerated. Immediately before each experiment, the actual injection solution was prepared by mixing 10 μl of the stock solution with an equal volume of 0·01 N NaOH. This solution contained 0·139 μCi/μl of [^{14}C]-5-HT at a pH of approx. 5·0.

A 10-μl Hamilton syringe filled with the 5-ion solution was mounted with the plunger completely depressed on a variable speed micro-injection pump. The tip of the cannula was placed in a small volume of the radioactive solution, the pump turned on in reverse, and 6 μl of a volume of [^{14}C]-5-HT solution was drawn into the cannula.

Immediately before each micro-injection, flow at the cannula tip was verified by turning the pump on briefly in the forward direction. After the cannula was inserted into the intracerebral guide tube, 1·5 μl of solution was injected into the site over an interval of 45 sec. To permit diffusion of the injected solution,[30] the cannula was left in place for 1 min after the end of each injection. Injections were made sequentially in the same order throughout the experiment into the caudate nucleus, lateral hypothalamus and mesencephalic reticular formation.

Push–pull perfusion. The push–pull cannulae used in this study were a miniaturized modification of the design of Myers.[26] To construct each of the cannulae, an outer "pull" tube of 23-ga thin-wall stainless-steel, approx. 35 mm long, was soldered into an 18-ga thin-wall tube, 10 mm in length, so that the lumen of the pull opened a short distance inside the larger tube. Second, the inner "push" tube of 28-ga regular wall stainless-steel, 55 mm in length with one end beveled at a sharp angle, was passed through the outer tube so that only 1 mm of the beveled end projected from the free end of the outer. Third, the outlet portion of the pull tube was cut from standard 23-ga stainless-steel tubing; one end was beveled and this was passed into the chamber formed by the 18-ga tube. The chamber was then soldered closed, thus fixing the push tube and 23-ga needle in place. After assembly, each cannula was checked for unobstructed flow through both the push and pull tubes. The cannulae were autoclaved before use.

After the first micro-injection, a period of 30 min preceded the beginning of the simultaneous push–pull perfusion of the three sites, since two previous investigations in this laboratory have shown that at least 90 per cent of a dose of [^{14}C]-5-HT microinjected into the rat brain is metabolized within 1 hr.[31,32] Each of the push and pull

tubes of the three canulae was connected via PE tubing to a 2·0-ml glass syringe mounted on a Harvard variable speed, infusion–withdrawal pump. Each push syringe was filled with the ion solution and the pump was turned on briefly to verify the pathway of the flow through the cannulae. After the cannulae were inserted through the guides into the appropriate sites in the rat's brain, the perfusion was begun at a flow rate of 80 μl/min, for a total of 10 min, and monitored closely. If flow was irregular, that cannula was immediately withdrawn and the sample was not included in the subsequent assay. The pull syringe was cooled with ice during the perfusion.

Assay procedure. The total radioactivity in each sample was determined rapidly by taking a 1-min count of a 50-μl aliquot of the sample added to 15 ml of a scintillation solution consisting of equal parts of reagent-grade toluene and 2-ethoxyethanol and 0·8% (w/v) 2,5-diphenyloxazole (PPO). If the net count rate of any sample fell below 200 counts/min, it was not carried through the subsequent assay, since pilot assays had shown that this level of activity could not be analyzed readily. The determination of the relative proportion of each of the three radioactive constituents, 5-HT, 5-HIAA and 5-HTOL, was performed for each sample using thin-layer chromatography. Prior to each experiment, commercially prepared mylar-backed sheets of Silica gel G, 20 cm square (Eastman–Kodak), were activated by baking at 90–100° for a minimum of 1 hr. In a line 2·5 cm from the bottom and at least 2·0 cm from either vertical edge, five points 3·0 cm apart were marked lightly in pencil on the sheet. Using a 10-μl disposable capillary pipette, a solution of 9 ml of absolute ethanol and 1·2 ml of 1 N HCl containing approx. 1 mg/ml each of non-labeled 5-HT, 5-HIAA and 5-HTOL was applied to the five points. When its tip was touched lightly to the surface of the sheet, the filled pipette released about 1–2 μl of solution. This was repeated until the entire contents of the pipette were emptied, using a stream of nitrogen to dry the spot between applications. The chromatogram was then stored for 2 hr or less in a dessicator evacuated to 127 mm of mercury.

An aliquot of each perfusate was applied on one of the five spots on a chromatogram prepared as described above. The volume of perfusate applied was dependent on the count rate detected during screening. If it was above 500 counts/min, 25 μl were spotted, while if it was below that, 50 μl were spotted, using 25- and 50-μl capillary pipettes. The order in which the sample of each perfusate was spotted was always caudate, lateral hypothalamus, then mesencephalic reticular formation and the test tubes containing the perfusates were kept in a bath of ice water.

During the assays, one or two standard solutions containing a known quantity of [^{14}C]-5-HT were applied onto the same chromatogram as the samples of perfusate. One standard solution was prepared by adding the [^{14}C]-5-HT to 0·25 ml of the 5-ion solution and the other by adding the [^{14}C]-5-HT to a 0·25-ml aliquot of one of the samples of perfusate. These standards were run as controls for the amount of degradation which might occur in a sample while being spotted on the chromatogram. Since the standards were always the last solutions to be applied, the maximum degradation possible during analysis was thereby reflected.

The solvent system of Aures *et al.*[33] was used to separate the three fractions and consisted of ethyl acetate, *n*-propanol, and 10% ammonium hydroxide in a ratio of 4:3:1. The development was allowed to continue until the solvent front reached a height of between 10 and 12 cm, resulting in a mean R_f value of 0·17 for 5-HIAA, 0·49 for 5-HT and 0·91 for 5-HTOL. Visualization of the spots on the chromatogram

was achieved by exposure to the atmosphere for a minimum of 24 hr. This resulted in oxidation of the non-labeled compounds to colored substances which marked the location of the much less concentrated [^{14}C]-labeled compounds.

After localization of the spots, a uniform area surrounding each was defined as containing the major portion of radioactivity present in the form of that particular substance. The dimensions of this area were determined from detailed analysis of chromatograms of known quantities of [^{14}C]-5-HT. Each column of three spots and the origin was then separated from the rest of the chromatogram, and the Silica gel from each area was scraped into separate counting vials. The vials were then filled completely with the thixotropic gelling agent, Cab-O-Sil, and after the addition of 15 ml of scintillation solution, shaken by hand until uniformly suspended. Each sample was counted for 10 min in a Packard 3320 Tri-Carb liquid scintillation counter with the gain set at 6 per cent for the [^{14}C]-channel, having a window width of 50–1000 (arbitrary units), and at 1 per cent for the external standard channel, having a window width of 140–1000 (arbitrary units).

TABLE 1. PER CENT (±S. E.) OF THE TOTAL ACTIVITY RECOVERED IN THE FORM OF EACH METABOLITE AND THAT REMAINING AT THE ORIGIN IN CHROMATOGRAMS OF A KNOWN QUANTITY OF [^{14}C]-5-HT ADDED TO AN ALIQUOT OF 5-ION SOLUTION OF PERFUSATE AND RUN SIMULTANEOUSLY WITH EXPERIMENTAL SAMPLES

	Origin	5-HIAA	5-HT	5-HTOL	No. of determinations
5-Ion standards	2·65 ± 0·79	4·23 ± 1·50	92·77 ± 2·06	0·36 ± 0·19	8
Perfusate standards	9·70 ± 4·50	7·28 ± 2·56	82·52 ± 3·75	0·50 ± 0·20	9

An analysis of the known quantities of [^{14}C]-5-HT which were run simultaneously with the samples of perfusate on several different occasions indicated that a small amount of degradation did occur in the course of the assays. Table 1 presents the results of this analysis including the number of determinations made. Since the mean per cent of the total activity recovered as 5-HIAA was 7·28 ± 2·56, this post-collection degradation of the [^{14}C]-5-HT could in no way account for the levels detected in each rat. The average efficiency of recovery in this assay was found to be 73·52 ± 6·44 per cent.

After the perfusion experiments were completed, each rat was anesthetized with Nembutal and the ascending aorta perfused with 10% buffered neutral formalin containing 0·058 mM Na_2HPO_4 and 0·029 mM NaH_2PO_4. Each brain was removed carefully from the calvarium, and after fixation, was rinsed in flowing tap water for 1 hr and sectioned at 30 μ on a Lipshaw Cryostat. Sections showing each site of the [^{14}C]-5-HT μ-injections and subsequent push–pull perfusions were mounted on slides and stained according to the method of Klüver and Barerra.[34] Table 2 shows the stereotaxic coordinates, according to the atlas of Pellegrino and Cushman,[28] of the intended sites of perfusion in the caudate nucleus, lateral hypothalamus, and mesencephalic reticular formation, and those of the actual sites of perfusion in the individual animals.

TABLE 2. STEREOTAXIC COORDINATES* FOR THE INTENDED SITES OF PERFUSION AND THE ACTUAL SITES OBSERVED IN EACH ANIMAL AFTER HISTOLOGICAL PREPARATION OF BRAIN SECTIONS

		Caudate nucleus	Lateral hypothalamus	Midbrain reticular formation
Intended sites	AP	8·0	5·8	0·0
	L	3·0	1·8	1·9
	V	4·0	8·0	4·0
T-18			No histology	
T-21		8·4	6·0	0·2
		2·5	2·0	2·0
		5·5	8·5	5·0
T-22		8·4	5·8	0·2
		2·5	2·0	2·5
		7·0	9·0	5·0
T-5		Lesion at site	5·8	Unable to locate
			2·3	
			9·0	
T-6		8·2	5·6	Not perfused
		2·5	2·3	
		5·0	8·0	
T-7		8·6	6·8	0·4
		3·6	1·5	1·0
		6·0	9·5	4·5
TC-1		8·0	5·4	0·4
		3·5	1·8	1·9
		4·5	9·0	5·0
TC-2		Lesion at site	5·8	0·2
			1·7	1·9
			9·0	4·0
TC-3		8·0	5·8	0·2
		3·5	1·8	2·7
		6·0	9·5	7·0

* Coordinates determined from the atlas of Pellegrino and Cushman (1967).

RESULTS

After the acute administration of 6 g/kg of ethanol or the chronic daily consumption of an average of 2·60 ± 0·14 g of the drug, no significant increase in production of 5-HTOL from exogeneously administered [^{14}C]-5-HT was noted in any of the three CNS structures (Fig. 1). That fraction of the total activity constituted by 5-HTOL remained relatively low in all cases. Nevertheless, the production of that metabolite in the caudate nucleus of those animals forced to drink ethanol was significantly lower than that of the control ($t = 2{\cdot}28$, df $= 4$, $P < 0{\cdot}05$) or acute ethanol rats ($t = 2{\cdot}60$, df $= 4$, $P < 0{\cdot}025$; Table 3). However, the mean level of 5-HTOL detected in the lateral hypothalamus of the animals given a single dose of ethanol was slightly above that of the control animals, 7·82 per cent as opposed to 4·21 per cent, and this increase approached significance ($t = 1{\cdot}81$, df $= 5$, $P < 0{\cdot}10$).

The increased level of 5-HIAA detected in the perfusate from the caudate nucleus of the acute ethanol rats was significantly different from that of the control animals ($t = 3{\cdot}29$, df $= 5$, $P < 0{\cdot}01$), as was the decreased level of serotonin ($t = 3{\cdot}21$, df $= 5$, $P < 0{\cdot}025$; Table 3). The reduction of serotonin in the perfusate collected from the caudate of the acute ethanol animals was also significantly below the level detected in the perfusates from chronic ethanol animals ($t = 2{\cdot}52$, df $= 5$, $P < 0{\cdot}05$). With respect to the activity in the perfusates obtained from the lateral hypothalamus, only the reduced level of serotonin found in the group of rats given an acute

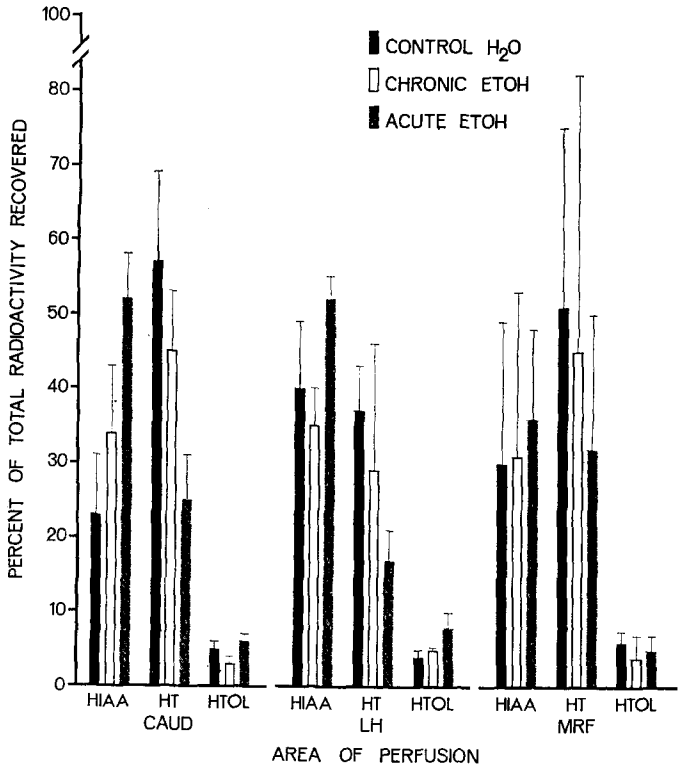

FIG. 1. Mean per cent of total carbon-14 radioactivity recovered (±S. E.) as 5-HIAA, 5-HT and 5-HTOL in the push–pull perfusates collected from each of the three anatomical locations in the brains of the controls, chronic forced ethanol drinkers, and acute ethanol animals.

dose of ethanol was significantly different than the control animals ($t = 3{\cdot}48$, df $= 5$, $P < 0{\cdot}01$). However a comparison of 5-HIAA levels in the perfusates obtained from this area in the chronic and acute ethanol animals revealed a significantly higher content in the latter group as opposed to the former ($t = 3{\cdot}68$, df $= 5$, $P < 0{\cdot}01$). No significant differences were observed in the relative percentages of the three metabolites detected in the perfusates from the mesencephalic reticular formation sites in the different groups of rats, though the pattern described previously still persisted.

The data obtained after a later replication of that portion of the study involving those rats which received 6 g/kg of ethanol provide an indication of the reliability

of the trends which were observed (Table 4). In all but one case, the ratio in the per cent of 5-HT to 5-HIAA which was observed initially was replicated in the direction of the change. The levels of the three metabolites detected in the perfusates collected from the mesencephalic reticular formation in these animals revealed a much greater interanimal variability than in the two other areas of the brain. In two animals a much greater percentage of 5-HIAA relative to 5-HT was found, but the other two showed the reverse trend for both the experiments in which the rats were perfused.

TABLE 3. MEAN VALUES FOR THE PER CENT OF THE TOTAL ACTIVITY IN THE PERFUSATE CONSTITUTED BY 5-HIAA, 5-HT AND 5-HTOL AT EACH OF THE THREE DIFFERENT ANATOMICAL LOCATIONS*

Anatomical locations	5-HIAA	5-HT	5-HTOL	Origin	No. of animals
Caudate nucleus					
Control	23·17	56·91	5·18	14·74	3
Chronic	33·90	44·98†‡	2·98§‡	18·14	3
Acute	51·68§‖	24·82§¶	6·39†¶	17·11	4**
Lateral hypothalamus					
Control	40·12	37·03	4·21	18·64	3
Chronic	34·81†‖	28·76	4·97	31·46	3
Acute	51·57	17·32§‖	7·82	23·29	4**
Midbrain reticular formation					
Control	29·95	50·67	5·67	13·71	2
Chronic	31·58	45·06	4·28	19·09	3
Acute	36·02	32·05	5·41	26·52	4**

* Significant comparisons are denoted by the superscript to the right of the appropriate value.
† Comparison between chronic and acute.
‡ $P < 0{\cdot}050$.
§ Comparison with control.
‖ $P < 0{\cdot}010$.
¶ $P < 0{\cdot}025$.
** One control rat served also for the acute replication.

The results obtained from rat T-21, which was perfused under both control and ethanol-sedated conditions, demonstrate the magnitude and direction of the change observed in the fate of exogenous 5-HT injected into the brain of an animal given an acute dose of ethanol (Fig. 2). The effect of ethanol is identical to that described between different animals in the two treatment groups; that is, ethanol greatly increases the per cent of exogenous 5-HT metabolized to 5-HIAA. This marked change is most apparent in the caudate perfusate, but was also present in the effluent collected from the hypothalamus, though to a lesser degree.

The actual amount of [^{14}C]-5-HT recovered in the push–pull perfusate from each animal was found to vary according to the area of the brain in which the cannula was located. A mean of $9{\cdot}3 \pm 1{\cdot}0$ per cent of the injected dose was recovered from the caudate nucleus, $3{\cdot}8 \pm 0{\cdot}6$ per cent from the hypothalamus and $2{\cdot}8 \pm 0{\cdot}5$ per cent from the reticular formation. The difference between the recovery from the hypothalamus and caudate nucleus was significant ($t = 4{\cdot}65$, $df = 23$, $P < 0{\cdot}005$), whereas that from the hypothalamus and reticular formation was not.

TABLE 4. INDIVIDUAL VALUES FOR THE PER CENT OF THE TOTAL ACTIVITY CONSTITUTED BY EACH METABOLITE AND THAT REMAINING AT THE ORIGIN FROM PERFUSIONS AT THREE SITES IN ANIMALS GIVEN 6 g/kg OF ETHANOL ON TWO OCCASIONS*

		Caudate nucleus		Lateral hypothalamus		Midbrain reticular formation	
	Rat	Acute No. 1	Acute No. 2	Acute No. 1	Acute No. 2	Acute No. 1	Acute No. 2
5-HIAA	TC-1	44·61	40·32	49·63	75·86	8·25	19·31
	TC-2	49·68	26·28	51·03	62·82	53·45	66·26
	TC-3	66·92	46·33	59·15		37·48	31·10
5-HT		31·42	42·73	20·68	2·59	72·68	61·38
		28·37	52·56	10·71	15·88	10·34	2·85
		10·28	31·92	12·42		42·12	50·00
5-HTOL		3·78	4·84	9·31	2·59	1·55	2·07
		5·91	1·07	4·78	4·33	4·89	3·25
		7·60	4·10	6·54		9·09	2·03
Origin		17·06	12·10	17·42	18·97	17·53	17·24
		16·05	20·09	33·49	16·97	31·32	27·64
		15·20	17·66	21·90		15·03	16·86

* The identification numbers of the three animals are shown in the upper left corner of the table and this order is the same throughout.

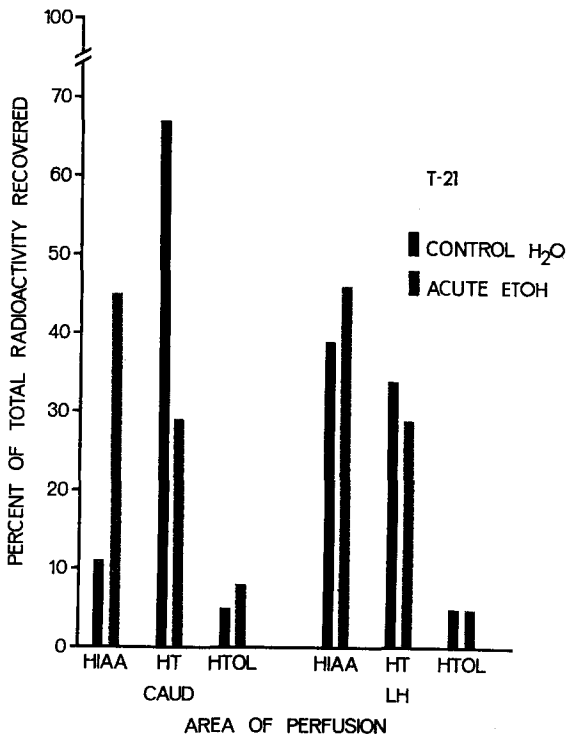

FIG. 2. Mean per cent of the total carbon-14 radioactivity recovered as 5-HIAA, 5-HT and 5-HTOL in the push–pull perfusates collected from two anatomical locations in rat T-21 under control and acute ethanol conditions.

DISCUSSION

Neither an acute dose nor chronic ingestion of ethanol was found to produce a substantial shift in the degradation of 5-HT from an oxidative to a reductive pathway. Similar observations have been reported on two previous occasions.[15,16] The discrepancy between these findings and others in which an increase in 5-HTOL production has been demonstrated[2,3,14,35–37] may be due to the failure to differentiate between 5-HT metabolized in the periphery and that metabolized centrally. That such a dichotomy exists is strongly supported by the finding that the incubation of rat brain slices in a medium containing ethanol failed to produce an increase in the amount of 5-HTOL detected, whereas liver slices incubated under similar conditions demonstrated a marked increase in 5-HTOL production.[15]

Additional evidence for the existence of differences between central and peripheral serotonin metabolism was provided most recently by Huff *et al.*[17] who showed that, in both control and ethanol-treated animals, the level of 5-HTOL detected is more than twice as high when [^{14}C]-5-HT is administered intravenously than when given intracranially. Nevertheless, even if ethanol would produce an elevation of 5-HTOL only in the periphery, it may still have a role in the production of CNS changes associated with ethanol intake, since 5-HTOL does in itself exert central effects.[38]

Ethanol caused an alteration in 5-HT metabolism in rats given a dose of 6 g/kg of ethanol 30 min before the injection of [^{14}C]-5-HT. The increase in the production of [^{14}C]-5-HIAA and concomitant fall in the amount of unchanged [^{14}C]-5-HT was greatest in perfusate collected from the caudate nucleus; a similar trend was detected in the perfusates from both the lateral hypothalamus and the midbrain reticular formation. These results are different than those observed by Huff *et al.*,[17] who analyzed the urine of ethanol-treated rats after an injection of [^{14}C]-5-HT into the lateral ventricle, and found a slight increase in the level of 5-HTOL but no change in the production of 5-HIAA. The discrepancies between these findings and ours may be due to the differences between the peripheral and central pathways of serotonin metabolism, as discussed previously. Our analyses were limited to the degradation of [^{14}C]-5-HT in the rat brain *in vivo*, whereas the examination of urinary metabolites[17] means that the [^{14}C]-5-HT, although administered in the brain, still must pass into the animal's peripheral circulation before being assayed.

The reason for the inconsistency between the results reported in this study and those in which no change or a slight increase was detected in the levels of 5-HT or 5-HIAA[6,16] probably can be accounted for by differences in procedure. Tyce *et al.*[16] and Palaić *et al.*[6] injected rats with different doses of ethanol and both groups of investigators analyzed whole brain levels of either injected or endogenous 5-HT. It is possible that whole brain levels of 5-HT may bear little or no relationship to the pattern of 5-HT metabolism which occurs in morphologically distinct regions of the brain of a conscious animal.[39]

Notwithstanding the procedural differences, one aspect of the findings of Palaić *et al.*[6] may have a direct bearing on our results, since they found that in response to acute ethanol the subcellular distribution of 5-HT in the brain shifted to twice the amount normally present in the unbound form. Since ethanol could thus release endogenous 5-HT from its storage sites, a possible explanation is provided for the higher levels of 5-HIAA seen in the acute ethanol animals. The ability of the cells at the different sites to bind the exogenous [^{14}C]-5-HT may have been reduced by

ethanol, thus leaving a greater portion of it available for enzymatic degradation to [^{14}C]-5-HIAA.

In the rats forced to drink an 8% solution of ethanol over a long period, no major effects on 5-HT metabolism, at least in those sites examined, were observed. In the perfusates from the caudate nucleus and midbrain reticular formation, the levels of [^{14}C]-5-HT and [^{14}C]-5-HIAA detected were always between those of the control and the acute ethanol animals. The level of [^{14}C]-5-HIAA in perfusates obtained from the lateral hypothalamus, however, was slightly lower than the control, but the amount of [^{14}C]-5-HT was between that of the control and the acute ethanol rats. It may be that this pattern is an indication of partial compensation at the cellular level for the 5-HT releasing effect of ethanol which was developed by the chronic ethanol rats over the long period of time that they drank this fluid. On the other hand, the pattern could be explained in terms of a dose–response relationship, since the amount of ethanol ingested by each rat just prior to a perfusion experiment was less than the single dose of ethanol given to the acutely treated rats.

It is unlikely that the differences in caloric intake between the chronic ethanol and the control and acute ethanol animals were of sufficient magnitude to be responsible for the changes in 5-HT metabolism. When given a choice of foods of different caloric or nutritional content, the rat has been shown to be quite adept at regulating its consumption of each so as to maintain a balanced diet.[40–42] A comparison of the mean daily food and fluid intakes, computed every 10 days, of the animals given malt-flavored ethanol to drink and the control animals, revealed that the former consumed slightly less solid food than the latter, $15{\cdot}7 \pm 0{\cdot}4$ g as opposed to $17{\cdot}3 \pm 0{\cdot}6$ g ($t = 2{\cdot}35$, df $= 24$, $P < 0{\cdot}025$), whereas both ingested similar volumes of fluid. In addition, there was no significant difference between the two groups of rats in their mean change in body weight, computed every 10 days, for the duration of the study. Thus, in spite of the different diets, the caloric intake of each group of animals was equivalent.

The fate of 5-HT injected into the brain may be difficult to specify, since catecholamine-containing neurons are capable of binding exogenous 5-HT.[43] Indeed, the observed difference in the quantities of radioactivity recovered from the caudate nucleus and the two other sites of injection emphasizes this question and the need for cautious interpretation of the data. Nevertheless, the level of [^{14}C]-5-HTOL should probably have increased regardless of the fate of the injected [^{14}C]-5-HT if the hypothesized shift in the ratio of NAD to NADH had occurred in the brain.[2,3,15] Additional experiments using a precursor of 5-HT as well as other biogenic amines will help to elucidate the complex relationships between ethanol and its effects on different structures in the central nervous system.

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