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EFFECTS OF PSYCHOACTIVE DRUGS ON THE METABOLISM OF INTRACISTERNALLY ADMINISTERED SEROTONIN IN RAT BRAIN

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Abstract—The effects of various psychoactive drugs on the metabolism of radioactive serotonin administered intracisternally were examined in rat brain. More radioactive serotonin was retained in the brains of rats anesthetized with pentobarbital during the intracisternal injection than in rats anesthetized with ether. Prior treatment with pargyline increased levels of intracisternally administered serotonin-^{3}H and decreased its deaminated metabolites, whereas reserpine decreased levels of serotonin-^{3}H. Imipramine, but not desmethylimipramine, slowed the disappearance of radioactive serotonin from brain. Chlorpromazine increased, whereas LSD decreased, the levels of radioactive deaminated metabolites of previously injected serotonin-^{14}C in brain. When lithium chloride was administered after the intracisternal injection of serotonin-^{14}C, levels of radioactive serotonin and deaminated metabolites in brain were increased above control values. The findings are discussed in relation to studies of the actions of these drugs on the metabolism of endogenous serotonin in brain, and to previous studies of the effects of these drugs on the metabolism of intracisternally administered norepinephrine.

MANY studies during the past decade have examined the effects of various psychoactive drugs on the metabolism of endogenous serotonin in animal brain *in vivo*.[1] More recently, radioactive serotonin has been used to study the effects of psychoactive drugs on serotonin uptake into brain tissue *in vitro*, either in brain slices or synaptosomal preparations.[2–5]

Techniques for administering radioactive biogenic amines into the lateral ventricles or basal cisterns of the brain, thus bypassing the blood-brain barrier, have facilitated the study of the effects of psychoactive drugs on the uptake, turnover and metabolism of monoamines in the brain *in vivo*.[6–8] While norepinephrine has been studied much more extensively with these techniques, the effects of drugs on serotonin metabolism have been examined by several investigators.[9, 10]

In the present study the effects of various psychoactive drugs on the metabolism of intracisternally administered radioactive serotonin have been studied in rat brain *in vivo*.

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METHODS

Sprague–Dawley rats weighing 180–200 g were injected intracisternally, as described elsewhere,[7] with 25 μl of Elliott's "B" irrigating solution (Baxter's) containing radioactive serotonin. Either serotonin-^{3}H creatinine sulfate, 1–3 μc (2·1 c/mM, Volk Radiochemical Co.) or serotonin-3′-^{14}C creatinine sulfate, 75–300 mμc (39·6mc/mM, Nuclear Chicago) were used in these experiments as indicated in the legends to the tables and figures. Animals were anesthetized lightly with ether during the intracisternal injections, and appeared to have recovered from the effects of the ether within 3 min. (In the experiments described in Table 2, pentobarbital was used as the anesthetic agent in one group of animals.) At various times after the intracisternal injections, groups of animals were killed by cervical fracture and decapitated. Whole brains, including 3–5 mm of spinal cord, were removed, rapidly rinsed three times with water and immediately homogenized in 10 ml of cold 0·4 N perchloric acid in an all-glass homogenizer. In some experiments, the brains were first frozen in liquid nitrogen before homogenization. The homogenates were centrifuged at 30,000 *g* for 30 min. In the experiments described in Table 1, the brains were decorticated. The cerebellum, pons-medulla and hypothalamus were removed as described by Glowinski and Iversen.[11] The remainder of the brain was assayed as a unit and is identified by the term "midbrain" in Table 1.

TABLE 1. RELATIVE CONCENTRATION OF SEROTONIN-^{3}H IN BRAIN REGIONS AFTER INTRACISTERNAL INJECTION*

Region	Relative concn. $\frac{\text{Serotonin-}^3\text{H/g of tissue}}{\text{Serotonin-}^3\text{H/g of cortex}}$
Cortex	1·0 ± 0·1
Midbrain	5·5 ± 0·5
Hypothalamic area	11·3 ± 1·5
Pons-medulla	8·9 ± 1·2
Cerebellum	8·5 ± 0·9

* Serotonin-^{3}H was injected into the cisterna magna of six rats. The mean serotonin-^{3}H content of the various regions of brain 1 hr later are expressed as the concentration relative to the cortex: $\frac{\text{Serotonin-}^3\text{H/g of tissue}}{\text{Serotonin-}^3\text{H/g of cortex}}$ ± S.E.M.

Assay of radioactivity. After centrifugation, an 0·3-ml aliquot of the supernatant fluid was added to 15 ml of a toluene-ethanol solution (10:4) containing phosphor, and the total radioactivity was assayed in a liquid scintillation spectrometer. Internal standards of toluene-^{3}H or toluene-^{14}C were used to correct for efficiency of counting.

Assay of radioactive serotonin. An 8-ml aliquot of the supernatant fluid was adjusted to pH 3 and passed through a 0.3 × 2 cm column of Dowex-50 (NH_4^+ form). After washing with 12 ml of water, the serotonin was eluted with 16 ml of an ethyl alcohol, 3 N HCl solution (3:1). Radioactivity in the eluate was determined as above.

Assay of radioactive deaminated metabolites. Effluents from the Dowex-50 columns (including the first 2 ml of the 12-ml water wash) were brought to a constant volume. Aliquots were adjusted to pH 1, salt saturated and extracted with 5 vol. of either ethyl acetate or diethyl ether. An aliquot of the organic phase was assayed for radioactivity.

RESULTS

Disappearance of serotonin-^3H from brain. After the intracisternal administration of serotonin-^3H, there was an initial rapid disappearance of this amine from the brain. The half-life of serotonin-^3H in the brain appeared to be 1·2 hr during the first 2 hr. At subsequent times, the rate of disappearance was considerably slower (Fig. 1).

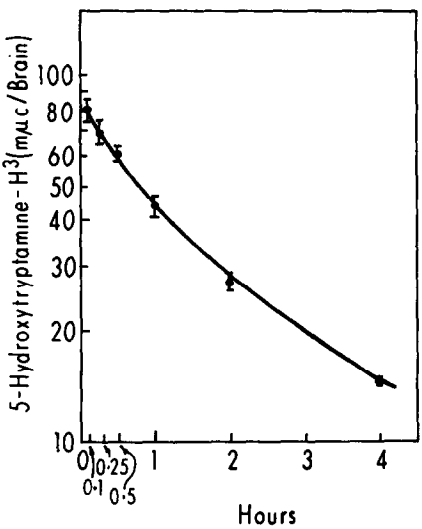

FIG. 1. Disappearance of 5-hydroxytryptamine-^3H from brain. Rats were injected intracisternally with serotonin-^3H. Groups of four to six animals were killed at various time intervals. Results (uncorrected for recovery) are expressed in mμc/brain, as the mean ± S.E.M. At 24 hr, 3·4 ± 0·1 mμc serotonin-^3H remained in the brain.

Relative concentration of serotonin-^3H in various brain regions. One hr after the intracisternal injection of serotonin-^3H, highest concentrations of radioactive amine were found in the hypothalamus which contained over eleven times as much tritiated serotonin per milligram of tissue as the cortex. Other regions examined contained a greater concentration of serotonin-^3H than the cortex, but less than the hypothalamus (Table 1).

Effects of pentobarbital anesthesia on the accumulation and disappearance of serotonin-^3H. Serotonin-^3H was administered by intracisternal injection to animals anesthetized either with ether or pentobarbital. Levels of serotonin-^3H were compared at various times after the intracisternal injection. At the earliest time examined (i.e. 0·1 hr), levels of tritiated serotonin were higher in the brains of animals injected under pentobarbital anesthesia than in animals anesthetized with ether. This difference in levels persisted over the 2-hr period studied. However, there appeared to be no significant difference between the groups in the rate of disappearance of serotonin-^3H from the brain, or in the ratio of tritiated deaminated metabolites to serotonin-^3H (Table 2).

Effects of psychoactive drugs on the disposition and metabolism of radioactive serotonin. Reserpine (5 mg/kg), pargyline hydrochloride (75 mg/kg), imipramine hydrochloride (25 mg/kg) or isotonic saline (control) was administered by intraperitoneal

injection* prior to the intracisternal injection of tritiated serotonin and animals were sacrificed 1 hr after the intracisternal injection. Compared to control values, levels of serotonin-^{3}H in brain were decreased in animals treated with reserpine, markedly increased in animals treated with pargyline and moderately increased in animals treated with imipramine. Tritiated deaminated metabolites in brain were markedly

TABLE 2. EFFECTS OF ANESTHESIA WITH ETHER OR PENTOBARBITAL ON THE DISAPPEARANCE OF SEROTONIN-^{3}H FROM BRAIN AFTER INTRACISTERNAL INJECTION*

Time (hr)	Ether		Pentobarbital	
	Serotonin-^{3}H	Tritiated deaminated metabolites	Serotonin-^{3}H	Tritiated deaminated metabolites
	(mμc/brain ± S.E.M.)		(mμc/brain ± S.E.M.)	
0·1	157 ± 10	37 ± 4	195 ± 11†	39 ± 3
0·5	93 ± 9	22 ± 2	120 ± 2†	30 ± 1†
1·0	67 ± 2	13 ± 1	95 ± 11†	21 ± 1‡
2·0	44 ± 4	9 ± 0·3	62 ± 3‡	13 ± 0·2‡

* Serotonin-^{3}H was injected into the cisterna magna of rats under ether or pentobarbital anesthesia. In animals anesthetized with pentobarbital, 50 mg/kg was administered intraperitoneally 30 min before the intracisternal injection. Groups of three animals were killed at the indicated times. Results (uncorrected for recovery) are expressed in mμc/brain as the mean ± S.E.M.
† $P < 0.05$ different from ether-anesthetized animals.
‡ $P < 0.01$ different from ether-anesthetized animals.

TABLE 3. EFFECTS OF PRETREATMENT WITH RESERPINE, PARGYLINE OR IMIPRAMINE ON THE METABOLISM OF SEROTONIN-^{3}H IN BRAIN*

Drug	Serotonin-^{3}H	Tritiated deaminated metabolites
	(mμc/brain ± S.E.M.)	
Saline	61·9 ± 1·5	11·5 ± 0·8
Reserpine	44·4 ± 2·3†	11·3 ± 0·9
Pargyline	169·3 ± 16·3†	1·8 ± 0·1†
Imipramine	75·2 ± 4·7‡	11·2 ± 0·8

* Reserpine (5 mg/kg) or pargyline hydrochloride (75 mg/kg) was administered i.p. 2 hr before the intracisternal injection of serotonin-^{3}H, or imipramine hydrochloride (25 mg/kg) was administered 1 hr before the intracisternal injection. Animals were killed 1 hr after the administration of serotonin-^{3}H and the brains analyzed for tritiated serotonin and deaminated metabolites as described in the text. Groups of 6 animals were used. Results (uncorrected for recovery) are expressed in mμc/brain as the mean ± S.E.M.
† $P < 0.001$ when compared with saline control valves.
‡ $P < 0.02$ when compared with saline control values.

decreased by pargyline; statistically significant changes in levels of radioactive deaminated metabolites were not observed with the other two drugs, but the ratio of tritiated deaminated metabolites to serotonin increased in animals treated with reserpine (Table 3).

When imipramine hydrochloride (25 mg/kg), desmethylimipramine hydrochloride

* See Table 3 for injection schedule.

(25 mg/kg), or chlorpromazine hydrochloride (25 mg/kg) was administered 90 min prior to the intracisternal injection of serotonin-^{14}C and animals were sacrificed 6 min later, a statistically significant difference in levels of serotonin-^{14}C in brain was not found between any of the drug–treated and control groups.

In another set of experiments, serotonin-^{14}C was first administered by intracisternal injection and various psychoactive drugs were subsequently injected intraperitoneally.* The animals were sacrificed 2 hr after the administration of serotonin-^{14}C. A significant increase in levels of serotonin-^{14}C occurred in the brains of animals treated with imipramine; statistically significant changes in serotonin-^{14}C levels were not observed with desmethylimipramine or chlorpromazine. Levels of radioactive deaminated metabolites in brain were significantly increased in animals treated with chlorpromazine. Lysergic acid diethylamide (LSD) caused a decrease in radioactive deaminated metabolites in brain, but levels of serotonin-^{14}C were not significantly different from control values. Levels of both radioactive serotonin and its deaminated metabolites were elevated in brains of animals treated with lithium chloride (Table 4).

TABLE 4. EFFECTS OF PSYCHOACTIVE DRUGS ON THE METABOLISM OF SEROTONIN-^{14}C IN BRAIN*

Drug (dose)	N	Serotonin-^{14}C	Radioactive deaminated metabolites
		% control ± S.E.M.	
Sodium chloride (isotonic)	21	100 ± 2	100 ± 3
Desmethylimipramine-HCl (25 mg/kg)	20	104 ± 3	101 ± 4
Imipramine-HCl (25 mg/kg)	21	120 ± 4†	109 ± 6
Chlorpromazine-HCl (25 mg/kg)	18	108 ± 4	112 ± 3‡
LSD (1·3 mg/kg)	19	102 ± 4	89 ± 4†
Lithium chloride (50 mg/kg × 2)	14	119 ± 6‡	123 ± 8§
Lithium chloride (200 mg/kg × 2)	14	119 ± 4†	132 ± 6†

* Serotonin-^{14}C was administered intracisternally. Desmethylimipramine, imipramine, chlorpromazine, lysergic acid diethylamide (LSD) or lithium chloride was injected intraperitoneally 15 min later. A second dose of lithium chloride was given 75 min after the intracisternal injection. All animals were killed 2 hr after the injection of the radioactive amine, and the brains analyzed for radioactive serotonin and deaminated metabolites. The number of animals in each group is given in the table (N). Results are expressed as per cent of saline control mean ± S.E.M. Control values (uncorrected for recovery): Serotonin-^{14}C = 8·2 mμc/brain; radioactive deaminated metabolites = 2·1 mμc/brain.

† $P < 0.001$ when compared with control valves.

‡ $P < 0.01$ when compared with control valves.

§ $P < 0.05$ when compared with control values.

DISCUSSION

One hr after intracisternal administration of tritiated serotonin, the amine is selectively retained in certain areas of the brain. The concentration of serotonin—^{3}H is highest in the hypothalamus and lowest in the cortex. The differential distribution of tritiated serotonin in the various regions of the brain examined in the present study, with the exception of the cerebellum, is in good agreement with the distribution of endogenous serotonin.[12] The high concentration of radioactive amine found in the cerebellum may be a function of the route of administration.

* See Table 4 for drug doses and injection schedule.

More of the serotonin-^{3}H appears to be retained in the brains of rats anesthetized with pentobarbital during the intracisternal injection than in rats anesthetized with ether. Similar effects of pentobarbital were previously observed when norepinephrine-^{3}H or urea-^{14}C was injected intracisternally, and it has been suggested that alterations in membrane permeability may account for these findings.[7]

The effects of pargyline, a monoamine oxidase inhibitor, and reserpine on the metabolism of intracisternally injected radioactive serotonin were consistent with the effects of these drugs on the metabolism of endogenous serotonin.[1] Pargyline produced the expected decrease in tritiated deaminated metabolites and increase in tritiated serotonin while reserpine caused a decrease in levels of serotonin-^{3}H and an increase in the ratio of tritiated deaminated metabolites to serotonin. The absolute level of tritiated deaminated metabolites, however, was not increased in animals treated with reserpine. Presumably this is a consequence of initial rapid metabolism of the injected serotonin-^{3}H and rapid removal of the tritiated deaminated metabolites from the brain. The continued accelerated depletion and metabolism of labelled precursor (serotonin-^{3}H) may lead to a decrease in both precursor and product (tritiated deaminated metabolites) but an increase in the product : precursor ratio.

Imipramine slowed the disappearance of radioactive serotonin from brain, but this effect was not observed with desmethylimipramine. Similar findings have been reported in studies of the effects of these drugs on the disappearance of endogenous serotonin from brain after inhibition of synthesis with α-propyldopacetamide, a tryptophan hydroxylase inhibitor.[13, 14] The disappearance of radioactive norepinephrine from the brain is also slowed by imipramine.[6, 8] Desmethylimipramine has been found to cause an even greater decrease in the rate of disappearance of intracisternally administered norepinephrine-^{3}H than does imipramine,[8] but in the present study desmethylimipramine did not alter the disappearance of radioactive serotonin from brain. Similarly, desmethylimipramine did not alter levels of tritiated serotonin in the brain after intraventricular administration of this amine.*

There appear to be some inconsistencies in the literature regarding the effects of imipramine and desmethylimipramine on the uptake of serotonin to brain, depending upon the technique used. When studied *in vitro*, both imipramine and desmethylimipramine have been observed to inhibit the uptake of serotonin into brain slices[2, 3] and isolated nerve endings.[4, 5, 15] In contrast, imipramine but not desmethylimipramine has been reported to inhibit the uptake of serotonin into brain *in vivo* after this amine was injected into the lateral ventricle.[16, 17] Furthermore, serotonin-^{14}C uptake *in vivo* was also not inhibited by desmethylimipramine in a study in which the ventricles were perfused with serotonin.[10] In the present study neither imipramine nor desmethylimipramine decreased the uptake of intracisternally administered radioactive serotonin in the brain. Since higher concentrations of serotonin were generally used in studies performed *in vivo* than in the studies done *in vitro*, it is possible that the effects of these drugs on specific transport mechanisms may have been masked by the entry of serotonin into the brain by nonspecific diffusion. It has been suggested recently that serotonin administered by intraventricular injection may be bound at sites different from those which contain endogenously formed serotonin.[18] Differences in drug doses, schedules of injection and routes of administration or differences in regions of brain examined in the various studies may also have contributed, in part,

* D. Eccleston, personal communication.

to the differences in findings. The possibility that there may be differences in the metabolism of serotonin *in vivo* and *in vitro*, which might account for some of the discrepancies in findings, must also be considered.

Levels of endogenous 5-hydroxyindoleacetic acid (5HIAA) in brain have been reported to be increased by chlorpromazine, but decreased by imipramine.[19] In the present study, the levels of radioactive deaminated metabolites in brain were elevated in animals treated with chlorpromazine; but no significant changes were observed in levels of radioactive deaminated metabolites in animals treated with imipramine or desmethylimipramine. Similarly, in a study in which radioactive serotonin was perfused through the lateral ventricle of rats, desmethylimipramine has been found to decrease levels of endogenous, but not radioactive 5HIAA in brain.[10] In another study, however, desmethylimipramine appeared to slow the release from brain of tritiated 5HIAA which had been administered by intraventricular injection.*

Imipramine, desmethylimipramine and a number of other tricyclic antidepressant drugs have been found to decrease the level of tritiated deaminated catechol metabolites of intracisternally administered norepinephrine-^{3}H in brain.[19a] Imipramine has also been found to decrease the urinary excretion of 3-methoxy-4-hydroxymandelic acid, the major deaminated metabolite of endogenous norepinephrine in man.[20]

The decrease in radioactive deaminated metabolites, without a change in the level of radioactive serotonin in animals treated with LSD, seems consistent with previously reported changes in the metabolism of endogenous serotonin after treatment with LSD. While an increase in endogenous serotonin in brain has been observed shortly after administration of LSD, at later times after LSD administration (as in the present study) endogenous serotonin levels were not different from control values, but endogenous 5HIAA levels (which undergo a phasic variation with the time after LSD administration) were decreased.[21]

The increase in levels of radioactive deaminated metabolites of serotonin in the brains of animals treated with lithium salts may be analogous to the increase in tritiated deaminated catechol metabolites of intracisternally administered norepinephrine-^{3}H observed after lithium chloride administration.[22] Lithium salts, however, appear to increase the turnover of norepinephrine in brain,[23, 24] whereas, in the present studies, lithium chloride administration slowed the disappearance of radioactive serotonin, suggesting that turnover may be decreased. It has been suggested that lithium salts may slow the rate of active transport of organic acids in both kidney and brain.[25] This could possibly account for the elevated levels of radioactive deaminated metabolites of serotonin found in the brains of animals treated with lithium chloride.

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* D. Eccleston, personal communication.

REFERENCES

1. S. GARATTINI and L. VALZELLI, *Serotonin*, p. 277. Elsevier, Amsterdam (1965).
2. K. J. BLACKBURN, P. C. FRENCH and R. J. MERRILS, *Life Sci.* **6**, 1653 (1967).
3. S. B. ROSS and A. L. RENYI, *Life Sci.* **6**, 1407 (1967).
4. C. D. WISE and H. W. RUELIUS, *Biochem. Pharmac.* **17**, 617 (1968).
5. T. SEGAWA and I. KURUMA, *J. Pharm. Pharmac.* **20**, 320 (1968).
6. J. GLOWINSKI and J. AXELROD, *Pharmac. Rev.* **18**, 775 (1966).
7. S. M. SCHANBERG, J. J. SCHILDKRAUT and I. J. KOPIN, *J. Pharmac. exp. Ther.* **157**, 311 (1967).
8. S. M. SCHANBERG, J. J. SCHILDKRAUT and I. J. KOPIN, *Biochem. Pharmac.* **16**, 393 (1967).
9. G. K. AGHAJANIAN, F. E. BLOOM, R. A. LOVELL, M. H. SHEARD and D. X. FREEDMAN, *Biochem. Pharmac.* **15**, 1401 (1966).
10. D. PALAIC, I. H. PAGE and P. A. KHAIRALLAH, *J. Neurochem.* **14**, 63 (1967).
11. J. GLOWINSKI and L. L. IVERSEN, *J. Neurochem.* **13**, 655 (1966).
12. L. VALZELLI and S. GARATTINI, *J. Neurochem.* **15**, 259 (1968).
13. H. CORRODI and K. FUXE, *J. Pharm. Pharmac.* **20**, 230 (1968).
14. H. CORRODI, K. FUXE and T. HOKFELT, *Eur. J. Pharmac.* **1**, 363 (1967).
15. J. M. DAVIS, R. COLBURN, D. L. MURPHY and W. E. BUNNEY, JR., in *Scientific Proceedings*, p. 228. American Psychiatric Assn. (1968).
16. K. FUXE and U. UNGERSTEDT, *J. Pharm. Pharmac.* **19**, 335 (1967).
17. A. CARLSSON, K. FUXE and U. UNGERSTEDT, *J. Pharm. Pharmac.* **20**, 150 (1968).
18. E. SANDERS-BUSH and F. SULSER, *Pharmacologist* **10**, 210 (1968).
19. M. DAPRADA and A. PLETSCHER, *J. Pharm. Pharmac.* **18**, 628 (1966).

19a. J. J. SCHILDKROUT, G. A. DODGE, and M. A. LOGUE, *J. Psychiat. Res*, in press.

20. J. J. SCHILDKRAUT, G. L. KLERMAN, R. HAMMOND and D. G. FRIEND, *J. Psychiat. Res.* **2**, 257 (1964).
21. J. A. ROSECRANS, R. A. LOVELL and D. X. FREEDMAN, *Biochem. Pharmac.* **16**, 2011 (1967).
22. J. J. SCHILDKRAUT, S. M. SCHANBERG and I. J. KOPIN, *Life Sci.* **5**, 1479 (1966).
23. H. CORRODI, K. FUXE, T. HOKFELT and M. SCHOU, *Psychopharmacologia* **11**, 345 (1967).
24. J. J. SCHILDKRAUT, M. A. LOGUE, and G. A. DODGE *Psychopharmacologia*, **14**, 135, 1969.
25. A. ANUMONYE, H. W. READING, F. KNIGHT and G. W. ASHCROFT, *Lancet* **1**, 1290 (1968).