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Regional Uptake of ^{3}H-Melatonin from Blood or Cerebrospinal Fluid by Rat Brain

MELATONIN (5-methoxy *N*-acetyltryptamine) is synthesized uniquely in the pineal glands of mammals through the action of the enzyme hydroxyindole-O-methyltransferase (HIOMT)[1,2]. Because the activity of this enzyme varies diurnally among rats kept in cyclic lighting conditions, it has been suggested that melatonin secretion follows a similar rhythmic pattern[3]. Melatonin has been identified in urine and in peripheral nerve[4]. Neither the kidney nor nervous tissue contains measurable HIOMT activity, however, and this has been taken as evidence that the indole is secreted from the pineal. It has not yet been possible to demonstrate melatonin in blood or cerebrospinal fluid (CSF); this inability may result from the absence of melatonin in body fluids, a very rapid rate of inactivation once the indole leaves the pineal, or the lack of sensitive techniques for assaying melatonin. For this reason, it is not known whether melatonin is normally secreted into the blood or the CSF.

Circulating melatonin enters the brain with little difficulty[5]. Intraperitoneal injections of melatonin increase the concentration of serotonin in midbrain and thalamus[6], and the hormone seems to produce at least some of its endocrine effects by acting on the brain[7]. It might thus be anticipated that the biological activity of a given amount of melatonin would be related to its ability to gain access to the central nervous system; this ability, in turn, might vary according to whether the material initially entered the blood or the CSF. We have examined the distribution of ^{3}H-melatonin in rat brain after injection into the blood or CSF.

Female Sprague–Dawley rats weighing 160–200 g were caged individually and kept in diurnal lighting conditions (lights on from 0600 h–1800 h) for 10 days before use. They were given free access to Purina chow and water. At 1000 h groups of six rats anaesthetized with ether received 0·774 μCi of ^{3}H-melatonin dissolved in 30 μl. of water into the right lateral ventricle[8]. Other groups of six animals received 5·14 μCi of ^{3}H-melatonin in a final volume of 200 μl. by injection into the tail vein. ^{3}H-Acetyl-melatonin with a specific activity of 200 μCi/μM was prepared as described before[9]. One hour after administration of melatonin the rats were decapitated and the brains divided into left and right halves. The left side was dissected into cerebral cortex, midbrain,

cerebellum, medulla–pons and hypothalamus[10]. The section labelled "midbrain" also contained the non-hypothalamic portion of the diencephalon. Each of these regions and the whole right half of the brain were assayed for ^{3}H-melatonin by extraction into chloroform[5].

After either the intraventricular or the intravenous administration of ^{3}H-melatonin, the compound was selectively concentrated within the hypothalamus and the brain stem; the ratio of hypothalamic to cortical ^{3}H-melatonin was about 3·2 : 1 after intraventricular injection of the indole, and 5·6 : 1 after its intravenous administration (Table 1). Because of its greater weight, the cerebral cortex retained a larger percentage of the ^{3}H-melatonin than any other brain region, but its concentration of the indole was lowest (Table 1).

More than 100 times as large a percentage of the administered dose of ^{3}H-melatonin was retained in whole brain, cortex and midbrain after its intraventricular administration than after its intravenous injection. This difference was forty-fold in the cerebellum and the medulla–pons.

^{3}H-melatonin is thus selectively localized within certain brain regions, and a much larger fraction of the administered material remains in the brain after its placement in the CSF than when it is injected into the blood. If melatonin secreted from the pineal can be assumed to behave similarly, it seems that its release into the CSF would be far more efficient in influencing cerebral structures than its secretion into the blood.

The selective concentration of ^{3}H-melatonin by the hypothalamus and midbrain is of interest in view of the fact that the indole seems to produce important biochemical and neuroendocrine effects on these regions. Intraperitoneal injections of melatonin have been shown to increase the levels of serotonin in midbrain and hypothalamus, and melatonin implants in the hypothalamus and midbrain modify the content of pituitary LH in castrated rats[7]. It may be of interest to determine whether

Table 1. REGIONAL DISTRIBUTION OF ^{3}H-MELATONIN IN RAT BRAIN

Region	Weight (mg)	Percentage of administered ^{3}H-melatonin retained (1×10^{-3}) Route of administration		Concentration of ^{3}H-melatonin ($\mu\mu$Ci/g) Route of administration	
		Intra-ventricular	Intra-venous	Intra-ventricular	Intra-venous
Whole brain	1,786 ± 40	448·4 ± 96·1	2·17 ± 0·30	2,034 ± 511	66 ± 12
Cortex	1,092 ± 30	168·2 ± 59·6	1·15 ± 0·18	708 ± 168	55 ± 7
Midbrain	260 ± 20	69·0 ± 13·4	0·63 ± 0·14	1,814 ± 293	137 ± 38
Cerebellum	284 ± 12	47·6 ± 3·0	0·82 ± 0·53	1,284 ± 128	128 ± 77
Medulla–pons	264 ± 20	42·5 ± 4·4	1·03 ± 0·49	1,199 ± 150	198 ± 94
Hypothalamus	80 ± 13	24·4 ± 4·4	0·44 ± 0·13	2,300 ± 511	309 ± 64

Groups of six rats received 0·774 μCi of ^{3}H-melatonin intraventricularly, or 5·14 μCi intravenously, and were killed 1 h later. The right side of the brain was assayed for ^{3}H-melatonin; the left side was dissected into various regions and assayed for ^{3}H-melatonin. Data are presented as mean ± standard error.

the cellular loci at which ^{3}H-melatonin is retained in the brain coincide with the sites of serotonin storage.

If the pineal normally secretes melatonin into the CSF, the dose of intraperitoneally or intravenously administered material needed to produce a given effect on the brain might be as much as 100 times greater than the amount of endogenous melatonin that normally produces this effect. Most of the published reports describing melatonin's endocrine effects have used intraperitoneal doses of 100–200 μg. This amount would probably be equivalent in its effects on whole brain to 0·5–1·0 μg of endogenous material secreted into the CSF.

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