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The pineal gland of the mole (*Talpa europaea*, L.) V) Activity of hydroxyindole-O-methyl transferase (HIOMT) in the formation of melatonin/5-methoxytryptophol in the eyes and the pineal gland

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Summary. Using a method in which no substrate is added, the capacity of HIOMT to synthesize melatonin and/or 5-methoxytryptophol has been determined in the pineal and the eyes of the mole, a mammal having an atrophied visual system. In all animals studied, the activity of HIOMT in both eyes taken together was 2-10 times higher than in the pineal. The results prove that the pineal is not the only, and not always the most important, source of methoxyindoles.

Introduction.

The pineal gland of all mammalian species so far examined is generally rich in serotonin. It also contains smaller quantities of related 5-hydroxy- and methoxyindoles (Quay and Halevy, 1962 ; Quay, 1963, 1974). Melatonin has aroused the greatest interest among scientists. Although present in nervous tissue, retina, Harderian gland, intestine, plasma and urine (Arendt *et al.*, 1975 ; Barchas and Lerner, 1964 ; Bubenik *et al.*, 1976*a*, *b*, *c*, 1977 ; Koslow and Green, 1973 ; Lynch *et al.*, 1975 ; Mull and Ralph, 1972 ; Pang and Ralph, 1975 ; Pang *et al.*, 1973, 1976, 1977 ; Pelham *et al.*, 1972, 1973 ; Raikhlin *et al.*, 1975 ; Rollag and Niswender, 1976 ; Vaughan *et al.*, 1974, 1976), melatonin has always been considered to be essentially a pineal hormone.

In the pineal gland serotonin is converted into N-acetyl serotonin by N-acetylation (Weissbach *at al.*, 1960) while this product is converted by O-methylation (Axelrod

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and Weissbach, 1960) to melatonin. The two enzymes required for these conversions are N-acetyltransferase (NAT) and hydroxyindole-O-methyl transferase (HIOMT). By other pathways of serotonin metabolism, HIOMT is also involved in the formation of 5-methoxy-tryptophol (McIsaac *et al.*, 1965), a compound known to show physiological activity (McIsaac *et al.*, 1964 ; Balemans, 1974), 5-methoxyindole-3-acetic acid (Lerner *et al.*, 1959) and 5-methoxytryptamine (Quay and Smart, 1967).

Initially, HIOMT was thought to be present only in the pineal gland. Subsequently it was identified in the mammalian retina (Cardinali and Rosner, 1971, 1972 ; Quay, 1965) and in the Harderian gland (Vlahakes and Wurtman, 1972 ; Cardinali and Wurtman, 1972). The presence of HIOMT in the retina is of special interest. This enzyme has also been found in lower vertebrate pineals (Fenwick, 1970 ; Baker *et al.*, 1965) which, like the retina, contain photoreceptor cells. Moreover it is now proved (Pevet and Collin, 1976 ; Pevet *et al.*, 1977) that the pinealocytes of mammals belong to a sensory cell line that has evolved from sensory pineal photoreceptors of lower vertebrates during phylogenetic development (Collin, 1969, 1971). Considering that the pinealocytes did evolve from photoreceptor cells, it seemed of interest to study the capacity of HIOMT to synthesize melatonin and/or 5-hydroxytryptophol in the pineal and in the eyes of a mammal possessing an atrophied and rudimentary visual system under natural conditions. The mole (*Talpa europaea*, L.) is an appropriate animal for such an investigation.

Material and methods.

Eight moles of both sexes and of different ages were captured in Deux-Sèvres (France) on March, 22, 1977. Immediately after capture these moles were decapitated under ether anesthesia and the pineal and the eyes were quickly removed. The organs were then frozen, transported and conserved in liquid nitrogen until they were investigated.

The details of the method used to determine HIOMT activity are extensively reported and discussed elsewhere (Balemans *et al.*, submitted for publication).

Using this method, the methylation of several 5-hydroxyindoles in the pineal and in the two eyes was analysed by using the 5-hydroxyindoles present in the tissue studied as a substrate. This implies that *no* extra substrate was added to the incubation medium (20 µl of 0.1 M phosphate buffer, pH 8.0 and 10 µl of S-adenosylmethionine-^{3}H, 1.5 µCi/10 µl in H_2SO_4, pH 2.5). After incubation at 37 °C for 60 min, thinlayer chromatography was performed with pineal tissue together with the incubation medium. The chromatograms were developed in chloroform : methanol : ammonia 25 p. 100 (60 : 35 : 5). The spots were scraped and counted (liquid scintillation counter Mark I of Nuclear-Chicago) in 75 µl ethanol and 10 ml of a scintillation liquid (toluene 1 000 ml POPOP 0.1 g ; PPO 5 g ; Cab-o-sil 40 g). With this technique, 5-methoxytryptophane, 5-methoxytryptamine, 5-methoxyindole-3-acetic acid can be separated as can melatonin and 5-methoxytryptophol which are located on the same spot. Blanks without pineals and/or eyes were included in each series of incubations (see Balemans *et al.*). The activity present on the identical spots, as the 5-methoxyindoles in the blanks, was subtracted from that of the tissues studied.

Results.

In the moles studied, the methylation of both N-acetylserotonin and 5-hydroxytryptophol was measured together (see Material and Methods). As no substrate was, added, the results indicate the presence of the 5-hydroxyindoles N-acetylserotonin and/or 5-hydroxytryptophol, the methylating enzyme HIOMT, and melatonin and/or 5-methoxytryptophol in the pineal and in the eyes.

The number of animals was too small to determine a possible difference in HIOMT activity in the methylation of N-acetylserotonin and/or 5-hydroxytryptophol between young and adult males and females. However, HIOMT activity in both eyes was always 2-10 times higher than that in the pineal (table 1).

TABLE 1

HIOMT activity in the formation of melatonin and/or 5-methoxytryptophol in the eyes and the pineal of the mole. The results are expressed in DPM (disintegration per min)

	Pineal	2 Eyes
young male.....	—	742
young male.....	609	811
adult male.....	609	1 840
adult male.....	452	4 934
young female.....	406	1 704
female (not determined)...	331	3 082
adult female.....	159	918
pregnant female.....	847	2 295

Discussion.

Quay (1974) wrote : « It is probably safe to assume that melatonin occurring in these and other mammalian tissues and fluid (the peripheral nerves, blood and urine) is derived from the pineal gland, because of the pineal localization of the enzyme responsible for the last step in melatonin's biosynthesis, and because of the above cited loss of melatonin from chicken serum after pinealectomy. There is no evidence as yet that the low levels of this enzyme, hydroxyindole-O-methyltransferase, detectable in retina and Harderian gland can contribute to a release of melatonin from these other tissues. » Bubenik *et al.* (1976*a*, *b*, *c*, 1977) however, on the basis of immunocytological investigations, concluded that the pineal was probably not the only source of methoxyindoles. The results obtained in the mole demonstrate clearly that in a mammal, an extra-pineal biosynthesis of methoxyindoles indeed occurs. Moreover, the results also prove for the first time that the pineal is not always the organ in which most of this compound is synthesized. These observations seem to be of importance especially when considering the paradoxical effects of melatonin reported by different authors. In mammals, melatonin is generally considered to

exert an antigonadotropic effect (for ref., see Kappers, 1976). Inhibition of the antigonadotropic influence of the pineal by this methoxyindole has, however, also been observed (Reiter *et al.*, 1975). Moreover, although many reports assume or imply only direct effects of administered melatonin, the nervous system and humoral routes are gradually receiving more attention. Quay (1974) and Pavel *et al.* (1973) suggested that melatonin may act within the pineal gland itself to influence the secretion of pineal antigonadotropic compounds (peptides or polypeptides ?). On the other hand, it has also been demonstrated (Hoffman and Küderling, 1977 ; Turek, 1977) that, after pinealectomy, an antigonadotropic activity of exogenous melatonin is observed. This proves that this antigonadotropic effect was not due to any change in synthesis of pineal peptides or any other compounds secreted by the pineal.

The production of methoxyindoles in different organs, as has now been demonstrated in the mole, could perhaps help in understanding the paradoxical endocrinological effects of melatonin. The methoxyindoles produced by the pineal cells could possibly act in these cells, while those produced by other organs could act via the bloodstream either on the pineal or on its target organs. Another possibility is that the methoxyindoles synthesized in the pineal may differ from those synthesized at other sites.

With the technique used, it has been impossible in the mole to separate the action of HIOMT in forming melatonin from that of its 5-methoxytryptophol-forming role. Other investigations, now in progress, will permit to determine whether there is a possible difference between the eyes and the pineal in the quantitative distribution of these two methoxyindoles.

As a high HIOMT activity has been demonstrated in the atrophied eyes of the mole, the question arises whether this observation can be related to the fact that the mole retina is rudimentary. Only future research with other fossorial blind mammals can answer this question.

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Résumé. L'activité de l'HIOMT dans la synthèse de la mélatonine et/ou du 5-méthoxytryptophol a été étudiée dans la pinéale et les yeux de la Taupe, un Mammifère possédant un appareil visuel atrophié. Dans tous les spécimens étudiés, il a été démontré que l'activité de l'HIOMT dans les yeux était de 2 à 10 fois supérieure à celle observée dans la pinéale. Ces résultats qui prouvent que la pinéale n'est pas toujours le seul et le plus important lieu de synthèse des méthoxyindoles, ont été discutés.

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