Brain Receptors Sensitive to Indole Compounds: Function in Control of Luteinizing Hormone Secretion

Abstract. *The placement of melatonin and of 5-hydroxytryptophol in the median eminence of castrated male rats is followed 5 days later by a significant decrease in pituitary stores of luteinizing hormone. Pituitary reserve of this hormone is also depleted after the implantation of melatonin, 5-hydroxytryptophol, and 5-methoxytryptophol in the reticular formation of the midbrain. It is suggested that these indole compounds, which are normally synthesized in the pineal gland, may intervene in the control of the secretion of luteinizing hormone, possibly by acting on specific receptors localized in the median eminence and in the midbrain.*

The pineal gland participates in the control of several reproductive processes (*1*); this gland is the only structure of the mammalian body which synthesizes melatonin (5-methoxy-*N*-acetyl-tryptamine), a compound which mimics several of the effects of pineal extracts and which is believed to be the pineal hormone (*2*). The systemic administration of melatonin to normal male and female rats significantly inhibits the secretion of the luteinizing hormone (LH) (*3*), the pituitary principle specifically involved in inducing ovulation in mammals (*4*). The implantation of fragments of pineal gland or of micro amounts of melatonin into the median eminence of the hypothalamus or into the reticular formation of the midbrain of castrated male rats significantly reduces plasma concentrations and pituitary stores of LH (*5*); these experiments suggest the possibility that these two areas of the central nervous system (CNS) contain receptors sensitive to changing concentrations of melatonin and that these receptors are of some importance for the regulation of LH secretion (*5*). In addition to melatonin, the pineal gland contains or synthesizes (or both) a few other indoleamines, for example, serotonin or 5-hydroxytryptamine (5-HT), 5-hydroxytryptophol (5-HTPH), and 5-methoxytryptophol (5-MTPH) (*6*). Some of these compounds share part of the endocrine effects of melatonin. When injected into the third ventricle of immature female rats, 5-HT inhibits the secretion of gonadotropins and retards puberty (*7*); when given systemically 5-MTPH reduces ovarian weight and the number of estrous smears in maturing animals (*6*) and blocks copulation-induced release of gonadotropins in adult rabbits (*8*). After systemic administration 5-HTPH is apparently inactive (*8*). We have studied the effects exerted on pituitary LH secretion by 5-HT, 5-HTPH, and 5-MTPH implanted into the two brain regions which are sensitive to melatonin. Cannulas bearing crystals of each of these compounds on their tips were implanted by means of a Stoelting stereotaxic instrument and of Krieg's atlas (*9*) into the median eminence (ME), the lateral reticular formation of the midbrain (midbrain), the frontal cerebral cortex, or the pituitary gland of castrated Sprague-Dawley male rats. The cannulas were fixed to the skull with dental cement. Immediately before implantation 5-HT, 5-HTPH, and 5-MTPH were mixed with solid cocoa butter; cocoa butter was chosen as the incorporating material because its melting point (35°) is close to body temperature, and because it is not irritating to neural tissue (*10*). Stainless steel tubes (20-gauge) were tamped into the mixture of cocoa butter plus the compound to be tested; excess material remaining on the external surface of the tubes was removed. In control experiments (sham-implanted animals) needles containing only cocoa butter were used. All implants were performed 3 weeks after castration; the animals were killed with a guillotine 5 days after the hormones were implanted. The anterior pituitary lobes of each group of animals were pooled (minimum four glands) and homogenized; their LH content was assayed by the ovarian ascorbic acid depletion method of Parlow (*11*) as modified by Schmidt-Elmendorff and Loraine (*12*). Ovarian ascorbic acid was determined according to the procedure of Roe and Kuether (*13*). A 2 + 2 design was used against a standard of LH provided by the National Institutes of Health (NIH-LH-S11-Ovine). Four animals were used for each dose; the index of precision of the assay (λ) and the relative potency of the unknown samples were calculated by the methods recommended by Gaddum (*14*). Only assays in which λ was less than 0.18 were accepted. The differences between the means were statistically analyzed by means of Student's *t*-test; a probability of $P \leq .05$ was accepted as statistically significant.

Serotonin (5-HT) and 5-MTPH do not significantly modify pituitary stores of LH after implantation in the ME;

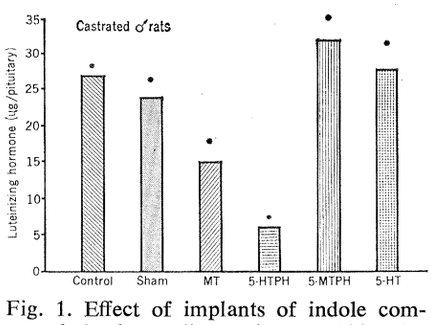

Fig. 1. Effect of implants of indole compounds in the median eminence. Abbreviations: MT, melatonin; 5-HTPH, 5-hydroxytryptophol; 5-MTPH, 5-methoxytryptophol; 5-HT, 5-hydroxytryptamine. Each column represents the mean (and the standard error) of three assays performed on three different pools of pituitary glands (four or five pituitaries per pool). Results are expressed as LH content per pituitary since there were no significant variations in pituitary weights in the different groups of animals. The difference between the MT group and either the control group or the sham-implanted group is significant at $P \leq .05$; the difference between the 5-HTPH group and either the control group or the sham-implanted group is significant at $P \leq .001$.

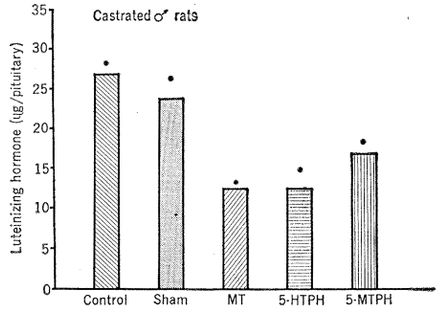

Fig. 2. Effect of implants of indole compounds in the reticular formation of the midbrain. Abbreviations: MT, melatonin; 5-HTPH, 5-hydroxytryptophol; 5-MTPH, 5-methoxytryptophol. Each column represents the mean (and the standard error) of three assays performed on three different pools of pituitary glands (four or five pituitaries per pool). Results are expressed as LH content per pituitary since there were no significant variations in pituitary weights in the different groups of animals. The difference between the MT and 5-HTPH groups and either the control group or the sham-implanted group is significant at $P \leq .02$; the difference between the 5-MTPH group and either the control group or the sham-implanted group is significant at $P \leq .05$.

on the contrary 5-HTPH left in the ME for 5 days induces a very significant ($P \leq .001$) decrease in the pituitary content of LH; the reduction in pituitary LH stores observed after placement of 5-HTPH in the ME is more pronounced than that obtained (*5*) after the implantation of comparable amounts of melatonin (Fig. 1). When placed in the reticular formation of the midbrain 5-HTPH was as effective as melatonin in reducing pituitary LH content (Fig. 2); the implantation of 5-MTPH in the midbrain resulted also in a significant decrease in pituitary LH stores. No one of the compounds tested was able to modify the pituitary reserve of LH when placed in the cerebral cortex or in the pituitary.

These data confirm that the CNS contains receptors which are sensitive to changing concentrations of indole compounds, and that these receptors may participate in the regulation of synthesis, or release of LH, or both. It is interesting to note that 5-HTPH, a compound which was believed to be inactive on endocrine phenomena (*8*), proved effective in modifying pituitary stores of LH after being placed in either the ME or the midbrain. It is quite possible that the activity of 5-HTPH was not discovered in previous experiments (*8*), because the compound cannot cross the blood-brain barrier when given systemically; of course this factor is not significant when the compound is implanted directly in the brain. It appears surprising that the receptors in the ME and those in the midbrain have a different sensitivity for the various compounds tested; ME structures are sensitive to melatonin and to 5-HTPH but not to 5-MTPH, whereas midbrain elements respond to melatonin, to 5-HTPH, and to 5-MTPH.

When placed in the ME, 5-HT was inactive in spite of the fact that it has been reported that 5-HT is metabolized to 5-HTPH by brain tissue (*15*); our results suggest that this conversion does not take place in the ME.

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Fluid Transport and Tubular Intercellular Spaces in Reptilian Kidneys

Abstract. *Renal tubules of crocodiles, lizards, snakes, and turtles have intercellular spaces similar in type to those observed in the mammalian gall bladder, but different from those of mammalian renal tubules. The fluid movements across renal tubules of reptiles are correlated with the width of the tubular intercellular spaces. In the proximal tubules, where transport is always isosmotic, the spaces are open whenever the tubular epithelium is tranporting, but closed when no transport is taking place. In distal tubules, intercellular spaces are wide open when the osmolality of the urine is close to that of the blood, that is, when the fluid resorbed is almost isosmotic to the tubular fluid. The apical two-thirds of the intercellular spaces are closed when the urine is hypoosmotic. They are also closed when the tubules are not transporting, as in collapsed tubules or tubules poisoned with ouabain. Thus, as in the gall bladder, the open intercellular spaces appear to be found whenever there is fluid transport across the epithelium.*

In the proximal renal tubules, as well as in the intestine and gall bladder of vertebrates, a single layer of epithelial cells transports salt and water isosmotically from the mucosal to the serosal side. Studies of gall bladder and intestinal epithelia in vivo and in vitro have shown that even when the mucosal fluid is hyperosmotic to the serosal fluid, transport of fluid may occur from the mucosal to the serosal side. This movement of water against its gradient was explained experimentally and theoretically as a solute-linked water transport involving a three-compartment system (*1*).

Electron microscopy of gall bladder fixed rapidly in osmium tetroxide or glutaraldehyde showed that an extra compartment does exist and that it appears to consist of the lateral intercellular spaces (*2*). The spaces were found to be wide open when fluid was transported across the gall bladder epithelium from mucosal to serosal side, but tightly closed when fluid transfer was inhibited in several different ways. Furthermore, the opening or closing of the spaces was not a result of swelling or shrinking of the cells.

Electron microscopy of the kidney of the crocodile *Crocodylus acutus* (*3*) showed open lateral intercellular spaces in the proximal and distal tubules, remarkably similar to those observed in the mammalian gall bladder. There were no basal infoldings, but there were thin, finger-like projections of the lateral membranes between the cells. This was surprising since teleost (*4*), amphibian (*5*), and mammalian kidneys (*6*) have proximal and distal tubular cells in which the cellular interdigitations give rise to the so-called basal infoldings.

These findings raised two major questions: First, do all reptilian kidneys show these characteristics of the lateral borders of the tubular cells, or do they differ with the renal adaptations to different habitats? Second, could the attractive hypothesis that the intercellular spaces are related to fluid transport be tested in reptilian kidneys by exposing the animals to various conditions that would further or stop fluid transport across the renal epithelium?

To answer these questions we studied the function and ultrastructure of the kidneys of reptiles from each of the four major groups of present-day living