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EVIDENCE FOR A FUNCTIONAL ROLE OF THE PINEAL IN BOVINES

M.M.M. Hayes, B.K. Knight and R.B. Symington

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Department of Anatomy, University of Rhodesia, Salisbury, Rhodesia

OPSOMMING: BEWYSE VIR 'N FUNKSIONELE ROL VAN DIE EPIFISE IN DIE BEES

Dat die epifise moontlik 'n regulerende invloed op die gonadotrofiese funksie van die adenohipofise uitoefen, is m.b.v. *in vitro* tegnieke ondersoek. Bykomstig, is 'n aaneenlopende stelsel ontwerp om die meganisme van die werking van die antigonadotrofiese beginsel van die epifise te bestudeer, aangesien daar 'n direkte negatiewe terugvoer van hiposifiële LH op die hipothalamus voorgekom het toe adenohipofisiale en hipothalamiese weefsel gesamentlik gekweek is. Resultate het aangetoon dat die hormone van die epifise die vrystelling van die tersake hipothalamiese vrystellingsfaktore inhibeer, waardeur die afskeiding van LH deur die adenohipofise verminder word. Laastens toon die resultate aan dat die epifise die produksie van LH-inhiberende faktore geproduseer deur die hipothalamus, stimuleer.

SUMMARY:

The possibility that the pineal exerts a regulatory influence on the gonadotrophic function of the adenohypophysis was investigated using *in vitro* techniques. Additionally, since a direct negative feedback of pituitary LH onto the hypothalamus occurred when adenohypophysial and hypothalamic tissues were incubated together, a continuous-flow system was devised to study the mechanisms of action of the pineal anti-gonadotrophic principles. Results demonstrated that the bovine pineal has marked anti-gonadotrophic properties *in vitro*. Data showed that the pineal hormones inhibited the release of the relevant hypothalamic release factor, thereby reducing secretion of LH from the adenohypophysis. Finally, results indicated that the pineal also stimulated the production by the hypothalamus of an LH-inhibitory factor.

From early times, many suggestions have been made regarding the function of the pineal. Thus, while Galen in 130 AD was of the opinion that, in man at least, the pineal was merely a vestigial organ, Descartes (1596–1650) contended that the pineal was the "seat of the soul". In recent years, it has been shown in laboratory species that the pineal has many effects including anti-gonadotrophic, anti-growth, anti-thyroid and anti-adrenal effects. (Reiter & Fraschini, 1968; Dickson & Hasty, 1972; Relkin, 1972; Simonnet, Thiéblot & Segal, 1951; Miline & Scepovic, 1959; Scepovic, 1963, and Singh & Turner, 1972). However, as yet, no specific function has been attributed conclusively to the pineal in higher animals.

Using chemical analysis of abattoir material, several workers have shown that the bovine pineal contains considerable amounts of indoleamines (McIsaac, Farrell, Taborsky & Taylor, 1965) and catecholamines (Giarman & Day, 1959 and Quay, 1963). Work with laboratory animals has shown that production of these substances by the pineal is related to, or affected by, stage of oestrous cycle, photoperiod, plane of nutrition, temperature and other environmental factors (Herbert, 1971; Nir, Hirschmann & Sulman, 1972). Recent results from both *in vitro* and *in vivo* studies suggest that an important effect of the pineal is its effect on the reproductive system (Benson, Matthews & Rodin, 1971; Fraschini, Mess & Martini, 1968 and Hayes, Knight & Warton, 1973). Further, it has been suggested that the pineal secretes its substances (hormones) into the cerebrospinal fluid (CSF) of the third ventricle and that these substances are extracted from the CSF by the modified ependymal cells (tanycytes) of the hypothalamus (Symington, Marks & Ryan, 1972). In turn, the pineal hormones act either directly or indirectly to modify the secretion of the trophic hormones of the anterior pituitary (Motta, Fraschini & Martini, 1967 and Moszkowska, Kordon & Ebels, 1971).

Evidence to support the antigonadotrophic role of the bovine pineal is presented in this paper.

Procedure

In general, definition, collection and preparation of tissues were as described by Symington & Hale (1972). Four types of experiments were conducted: *Groups 1, 2, 3 & 4.*

Group 1. Here, bovine stalk median eminence (SME), anterior pituitary (AP) and pineals (P) were incubated together in one flask. The incubation procedure was that of Hayes, et al (1973).

Group 2. Bovine SME was replaced by whole bovine hypothalami (H) which included the SME. Incubation was as for Group 1. These trials were conducted to determine whether the pineal acts directly on the AP, or indirectly via the hypothalamic release factors. Here, the inhibitory effect of the pineal on the AP alone was compared with its effect on the AP + H system.

Group 3. In these experiments, two different incubation techniques were followed viz. the continuous flow and recirculatory systems (Fig. 1). In both systems, bovine AP and H were incubated in separate flasks. In the continuous flow system, the effluent medium from the flask containing H was passed into the flask containing the AP. The effluent medium from this flask was collected continuously using a fraction collector (15 min. intervals). In the recirculatory system, the same procedure was followed except that

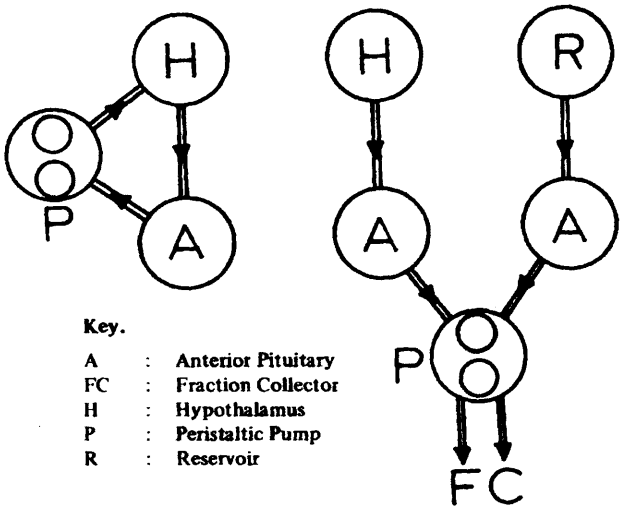


Diagram of the recirculatory and continuous flow systems of incubation

the effluent from the AP flask was recirculated into the H flask. The recirculated medium was collected at the end of the incubation period (4+hr).

Group 4. The continuous flow system of incubation was used. Bovine pineals were added to the basal system, i.e. AP alone, and to a system in which AP+H was used.

Subsequent to collection, the medium from all trials was stored at -20^{o} until assayed for luteinizing hormone (LH). This was done using the solid-phase radioimmunoassay procedure described by Ellison (1972). Radioactivity was assessed in a Packard Auto Gamma Spectrometer and data were processed on a Burroughs C 3600 computer. NIH-LH-B7 was used as the assay standard.

Results

Group 1. In a large number of trials, addition of bovine pineal to a tissue culture system containing bovine AP and SME has resulted in an appreciable decrease in the amount of LH released into the culture medium. Representative figures for these trials are shown in Table 1

Group 2. The results of these trials are shown in Table 2. Addition of pineal to either the AP alone or to the AP+H system decreased release of LH into the medium. It is of note that the pineal had a greater inhibitory effect on the AP+H system than it did on the AP alone. However, by comparison with the release of LH from AP alone, (basal release), addition of H to the incubation system failed to increase the amount of LH released into the medium. A comparison of addition of SME to the basal AP system with addition of H to this system is shown in Table 3.

Group 3. As a consequence of the results in the Group 2 trials, the possibility was investigated that there was a direct negative feedback of LH on the hypothalamus. These experiments necessitated the use of the continuous flow and recirculatory systems of incubation. Separation of H from AP in the continuous flow system considerably enhanced the release of LH into the culture medium (compared with the basal release). In marked contrast to this finding, recycling of the medium between the two flasks (AP+H) in the recirculatory system resulted in a release of LH which did not differ significantly from the basal release (Fig. 2).

Group 4. Preliminary studies have been carried out using the continuous flow technique described above. Addition of bovine pineal tissue to AP in the continuous flow system enhanced the release of LH into the culture medium. Addition of H to the AP considerably enhanced LH release. By contrast, inclusion of pineal in the AP+H system, resulted in a marked decrease in the quantity of LH released into the medium (compared with that from either the AP alone or that from AP+H) (Fig. 3).

Table 1

The effect on the release of LH of addition of pineal tissue to an in vitro *system containing bovine adenohypophysis and stalk median eminence*

	Concentration of LH in medium (ng./ml.) ± SE of mean	Relative efficiency of system (%)	Inhibition by Pineal (%)
AP+SME	4,97 ± 1,11	100,0	
AP+SME + P	3,99 ± 0,93	80,3	19,7

Key
LH : Luteinizing hormone: NIH – LH – B7 used as standard.
SME : Stalk median eminence.
H : Hypothalamus.
AP : Anterior Pituitary.
P : Pineal.
S.E. : Standard error

Table 2

The effect of incubation of bovine hypothalamus and/or AP with pineal on the release of LH

	Concentration of LH in medium (ng./ml.) ± SE of mean	Relative efficiency of system (%)	Inhibition by pineal (%)
AP alone	3,82 ± 0,67	100,0	–
AP + P	3,60 ± 0,58	94,1	5,9
AP + H	2,90 ± 0,62	75,9	–
AP + H + P	2,20 ± 0,53	57,6	24,2

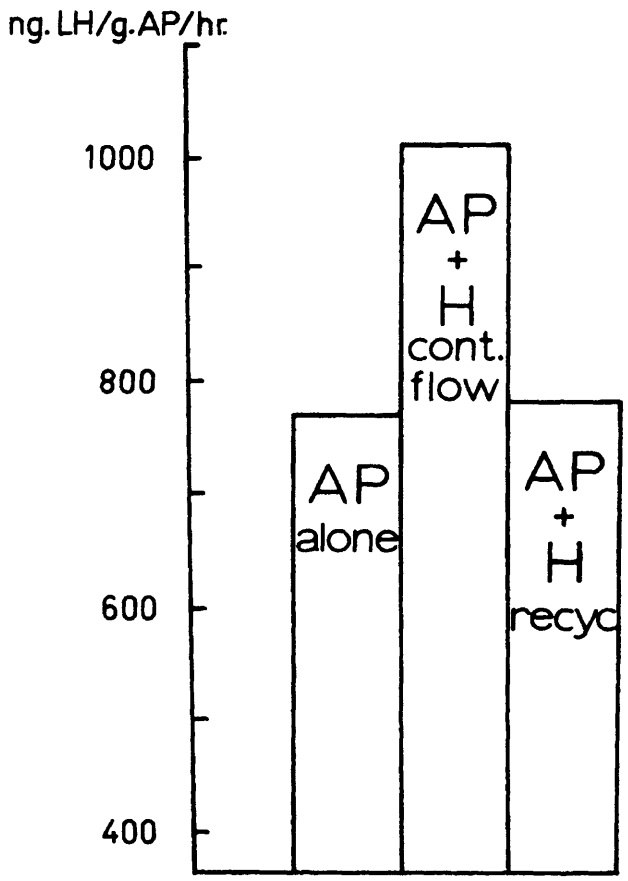
Key
LH : Luteinizing hormone: NIH – LH – B7 used as standard.
SME : Stalk median eminence.
H : Hypothalamus.
AP : Anterior Pituitary.
P : Pineal.
S.E. : Standard error

Table 3

Comparison of the effect on the release of LH of addition of SME or H to an in vitro *system containing bovine AP*

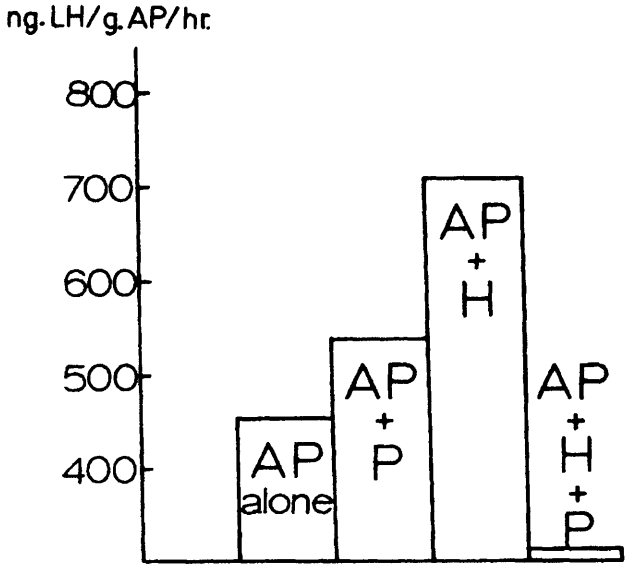
	Concentration of LH in medium (ng./ml.) ± SE of mean	Relative efficiency of system (%)
AP alone (in H trials)	3,82 ± 0,67	100,0
AP + H	2,90 ± 0,62	75,9
AP alone (in SME trials)	3,75 ± 0,65	100,0
AP + SME	6,90 ± 0,73	184,0

Key
LH : Luteinizing hormone: NIH – LH – B7 used as standard.
SME : Stalk median eminence
H : Hypothalamus
AP : Anterior Pituitary.
P : Pineal.
S.E. : Standard error



Least significant difference between treatment means: 152,0 (P< 0,0001)

Fig. 2. In vitro *demonstration of a direct negative feedback of pituitary LH onto the hypothalamus in the bovine*



Least significant difference between treatment means: 136,0 (P 0,001)

Fig. 3. *Group 4 experiments: The effect of the pineal on release of LH from the basal AP and the AP+H systems using the continuous flow incubation procedure*

Discussion

Although the role of the pineal as a neuroendocrine transducer has been established in laboratory species (Wurtman & Anton-Tay, 1969) its function in domestic and higher animals is still controversial. Thus, while melatonin and other indoleamines have been isolated from bovine pineal, (Lerner, Case, Takahashi, Lee & More, 1958 and McIsaac, *et al.*, 1965) and incriminated as the likely pineal hormones in laboratory animals (Fraschini, Collu & Martini, 1971) it is by no means certain that such amines play the entire, if any, endocrine role in higher animals. Indeed, the isolation of a peptid 8-arginine vasotocin from the bovine pineal with antigonadotrophic properties (Cheesman & Fariss, 1970) together with the work of Benson *et al.* in rats, suggests that the pineal could be operating through a group of peptid hormones (Benson, *et al.*, 1971). Again, the actual target organ of the pineal hormones and the route of the hormones to the target organ are also still conjectural. Further, much has still to be learned about the mechanism of action of the hormones at the target organ site. Several of these aspects of pineal function are being investigated currently in this laboratory. The present study was designed to show whether or not the bovine pineal had an anti-gonadotrophic influence *in vitro*, and if so, how this effect could be mediated. Results from the first group of trials (where pineal tissue was added to AP+SME maintained in the same flask), indicated that the bovine pineal has marked anti-gonadotrophic properties *in vitro*. However, the results cast no light on the route of action of the pineal hormones i.e. whether the hormones act directly on the anterior pituitary or indirectly on the hypothalamus. Results from the Group 2 experiments showed that the pineal was inhibitory to LH release in both the pineal +AP and the pineal +H+AP systems. That the pineal inhibited the AP+H system more than the AP alone system indicates that a gonadotrophin inhibitor substance is produced in the hypothalamus under stimulation from the pineal. Failure of the hypothalamus to enhance the basal release of LH as observed in the second group of experiments (AP+H), indicated that for some unknown reason, the entire hypothalamus functioned differently from the SME alone. Indeed, although not statistically significant, the data (Table 2) indicate that addition of hypothalamic tissue to adenohyphophysial tissue could depress release of LH.The possibility that such a depression was caused by a hypothalamic factor inhibiting release of LH is being investigated currently. In other words, using this system, it was impossible to determine whether the pineal acted on the pituitary directly or via the hypothalamus. Results from the third group of experiments demonstrated the presence of a direct negative feedback of pituitary LH onto the hypothalamus above the level of the SME. This finding explained the results obtained in the second group of experiments (where the H functioned differently from the SME). The possibility that the loss of LH from the medium was attributable to absorption and/or degradation of the LH molecule by the hypothalamic tissue was eliminated by trials where standard NIH-LH was incubated with hypothalami (Symington, Hayes, Knight & Hale, 1973).

Preliminary studies (Group 4 experiments) using the continuous flow techniques suggested that the pineal inhibited the release of gonadotrophin release factors from the hypothalamus. Thus, addition of pineal to the AP+H system markedly reduced the amount of LH released into the medium (compared with that released by the AP+H system itself). Additionally, that the release of LH from the AP+H+P system was less than that from AP alone indicates that the pineal stimulates production of a gonadotrophin-inhibitor substance from the hypothalamus. This view is supported by similar results in the Group 2 experiments. It is also of note that Fraschini *et al.* (1968). (Using the rather physiologically-suspect technique of implantation to investigate the possible inhibitory effects of catecholamines on release of LH) also postulated that the pineal hormone, melatonin, acted via the hypothalamus.

The present studies have shown that the bovine pineal has marked anti-gonadotrophic properties *in vitro* and that these properties are mediated through the hypothalamus. However, the entire role of the pineal in control of the reproductive activity of bovines is still to be elucidated. It could be that the pineal controls onset of puberty and oestrus in the bovine and that it is responsible in part at least for the influence of environmental stimuli (e.g. the photoperiod) on the reproductive state of cattle. Thus, the importance of the pineal in animal production at an economic level must not be neglected.

The few instances in all these studies where inclusion of pineal enhanced gonadotrophin secretation indicate the difficulties facing investigators in this field, and emphasise (a) the complexity of the problem and show how little is known about pineal function in the bovine and (b) the necessity for further investigations of the function of the pineal using both *in vivo* and *in vitro* techniques.

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