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A SYNERGISTIC INTERACTION BETWEEN HISTAMINE AND VASOACTIVE INTESTINAL PEPTIDE (VIP) ON CYCLIC AMP PRODUCTION IN THE CHICK PINEAL GLAND

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The effects of vasoactive intestinal peptide (VIP) and histamine, alone and in combination, on cyclic AMP formation have been studied in selected tissues of the central nervous system (CNS) of chick. VIP strongly stimulated cyclic AMP production in the pineal gland, moderately in the retina (maintained in "eye-cup" preparations), and had no effect in cerebral cortical slices. Combination of VIP with forskolin produced a synergistic response in the pineal gland; in the cerebral cortex VIP did not influence the elevation of cyclic AMP production evoked by forskolin, histamine and isoprenaline. Histamine stimulated cyclic AMP synthesis in the all tested CNS tissues, showing the following order of sensitivity to the amine: pineal gland > cerebral cortex > retina. The effects of histamine were stronger in the presence of forskolin. A combination of VIP and histamine produced the cyclic AMP response in the pineal gland clearly more than additive; such a synergistic interaction was antagonized by aminopotentidine, an accepted in mammals H_2-histamine receptor blocker. It is concluded that both VIP and histamine can be considered as functionally important neuromodulators in avians (similar to mammalian species). These two substances may play in concert to regulate the pineal physiology in avian species.

Key words: *chick, vasoactive intestinal peptide (VIP), histamine, cyclic AMP, pineal gland, central nervous system*

INTRODUCTION

Histamine is an established neurotransmitter/neuromodulator in the central nervous system (CNS) in many vertebrate species (1—3). Its biological activity, at least in mammals, is mediated via three histamine receptor subtypes, designated as H_1-, H_2-, and H_3-receptor (4). The main effector systems linked to the H_1- and H_2-type histamine receptor have been identified (phospholipase C and adenylyl cyclase, respectively), whereas the H_3-type histamine receptor-coupled effector system remains to be established.

Recently, we have reported that histamine is a potent activator of cyclic AMP synthesis in the pineal glands of chick, duck and goose (5—8). This finding, together with the ability of the chick pineal to synthesize and inactivate histamine (9), suggests that the amine may be of functional significance in the avian pineal gland. Yet, histamine, despite of its powerful action on cyclic AMP generation, only poorly affects secretion of the principal pineal hormone melatonin from organ-cultured chick pineal gland. This is in contrast to, e.g., vasoactive intestinal peptide (VIP), capable of stimulating effectively both cyclic AMP production and cyclic AMP-dependent melatonin biosynthesis in the chick gland (10, 11).

In the present study we asked whether histamine and VIP can interact with respect to cyclic AMP synthesis in the avian pineal gland. Such an information may be important in searching the role of both substances in the pineal physiology. For comparative purposes, we have investigated in addition the effects of VIP on cyclic AMP production in the chick retina and cerebral cortex.

MATERIALS AND METHODS

Experiments were carried out on 2—3-weeks old white male leghorn chicks (*Gallus domesticus*) kept from the day of hatching in warmed brooders with standard food and water available *ad libitum*, under a 12 h light/12 h dark lighting schedule (lights on between 22.00 and 10.00). Light intensity near the floor of the animals' room was about 150 lux. On the day of an experiment, the animals were killed by decapitation under standard laboratory illumination between 9.00—9.30, and their pineal glands were quickly isolated, and stored (until completion of the tissue collection) in cold, O_2/CO_2 (95:5)-gassed, glucose containing modified Krebs-Henseleit medium (KHM; mmol/L): 118, NaCl; 5, KCl; 1.3, $CaCl_2$; 1.2, $MgSO_4$; 25, $NaHCO_3$; 11.7, D-glucose; pH 7.4). Some experiments were carried out on cerebral cortex tissue, which following isolation, was rapidly cross-sliced (0.25 mm) with the aid of a McIlwain tissue chopper, and on retinal pieces, maintained in „eye-cup" preparations (each retina being divided into four parts, as described previously; 12).

Cyclic AMP assay

The synthesis of (^{3}H)cyclic AMP in the studied tissues prelabeled wth (^{3}H)adenine was determined by the method of Shimizu *et al.* (13). The formed (^{3}H)cyclic AMP was isolated by a sequential Dowex-alumina chromatography according to Salomon *et al.* (14). The results were individually corrected for a percentage recovery with the aid of (^{14}C)cyclic AMP added to each column system prior to the nucleotide extraction. The accumulation of cyclic AMP during a 15-min stimulation period (experiments with histamine, VIP, isoprenaline and forskolin) was assessed as a per cent of the conversion of (^{3}H)adenine into (^{3}H)cyclic AMP. Tissue samples were preincubated with aminopotentidine for 10 min prior to stimulation. Details of the whole procedure were described by us earlier (5, 6, 12).

Chemical compounds

The following drugs were used: histamine dihydrochloride (Serva, Heidelberg, Germany), forskolin, (R)(−)isoprenaline (isoproterenol) hydrochloride, and "mammalian" VIP (Sigma, St. Louis, MO, USA). Aminopotentidine was kindly donated by Prof. H. Timmerman (Vrije Universiteit, Amsterdam, The Netherlands). Radioactive compounds were (2,8-^{3}H)adenine (specific activity 26.9 Ci/mmol) and (^{14}C)cyclic AMP (specific activity 52.3 mCi/mmol; both from DuPont-NEN, Bad Homburg, Germany).

A short comment requires VIP that we used in the present study. The peptide is synthesized according to the mammalian formula (human, porcine, rat; Sigma V-6130). The mammalian VIP differs from the chick one in four amino acid residues (15), i.e. $Thr^{11} \rightarrow Ser^{11}$, $Leu^{13} \rightarrow Phe^{13}$, $Ile^{26} \rightarrow Val^{26}$, $Ans^{28} \rightarrow Thr^{28}$ (*Fig. 1*); this, however, does not seem to influence its biological activity in mammals and avians (10), although the mentioned four amino acid displacements in the two peptides may confer perhaps important antigenic differences (16, 17).

VIP (mammalian)

HIS-SER-ASP-ALA-VAL-PHE-THR-ASP-ASN-TYR-THR-ARG-LEU-ARG-LYS

GLN-MET-ALA-VAL-LYS-LYS-TYR-LEU-ASN-SER-LIE-LEU-ASN-NH^{2}

VIP (chicken)

HIS-SER-ASP-ALA-VAL-PHE-THR-ASP-ASN-TYR-SER-ARG-PHE-ARG-LYS

GLN-MET-ALA-VAL-LYS-LYS-TYR-LEU-ASN-SER-VAL-LEU-THR-NH$_{2}$

Fig. 1. The amino acid sequence of vasoactive intestinal peptide (VIP) from mammals (human, porcine, rat) and chickens. Note that the two peptides differ in four amino acid residues (underlined).

Data analysis

All data are expressed as mean ± SEM values. For statistical evaluation of results, ANOVA was used followed by the Newman-Keuls test.

RESULTS

VIP (0.001—1 μM) concentration-dependently and potently stimulated cyclic AMP formation in the pineal gland of chick; its effect was comparatively weak in the retina, and none in the cerebral cortex (*Fig. 2*). Combination of 0.1 μM VIP with 1 μM forskolin resulted in a synergistic "cyclic AMP"

response in the pineal gland (*Fig. 3*). However, a similar combination, extended also for a lower concentration of VIP (i.e. 0.01 μM), had no significant effect on the forskolin-evoked increase in cyclic AMP production in the cerebral cortex (*Table 1*). VIP (0.01—1 μM) applied simultaneously with isoprenaline (10 μM), or with histamine (1 and 100 μM), did not markedly modify the effects produced by these compounds in the cerebral cortex (*Table 1*).

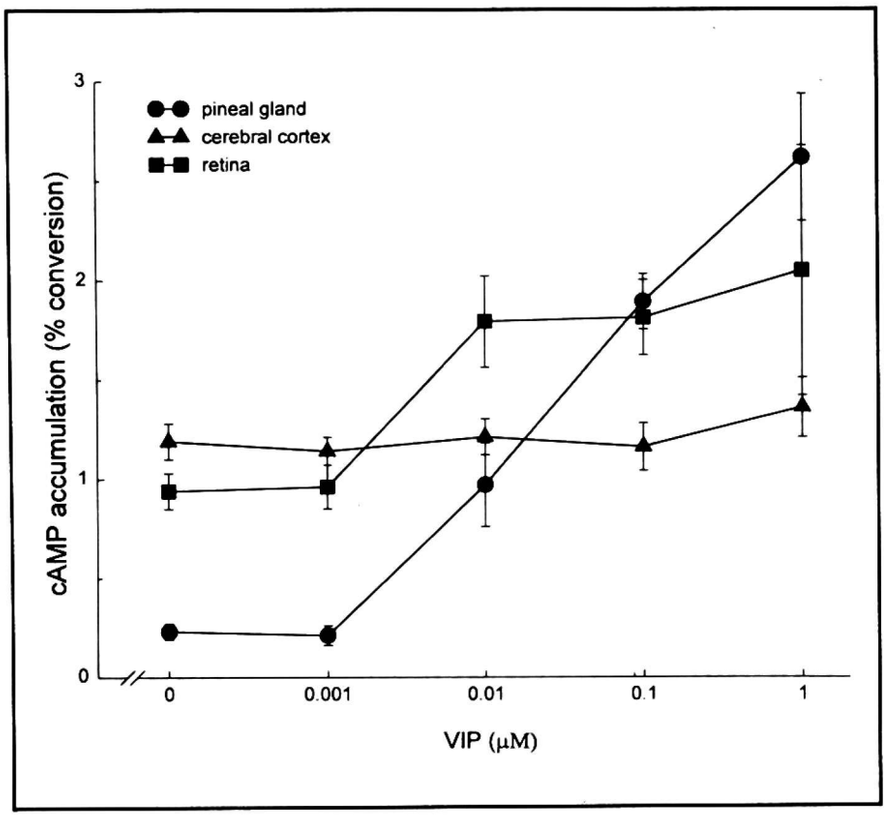

Fig. 2. The effects of vasoactive intestinal peptide (VIP) on cyclic AMP formation in the pineal, retina and cerebral cortex of chick. Results are means ± SEM from $n = 10$—31 assays/point.

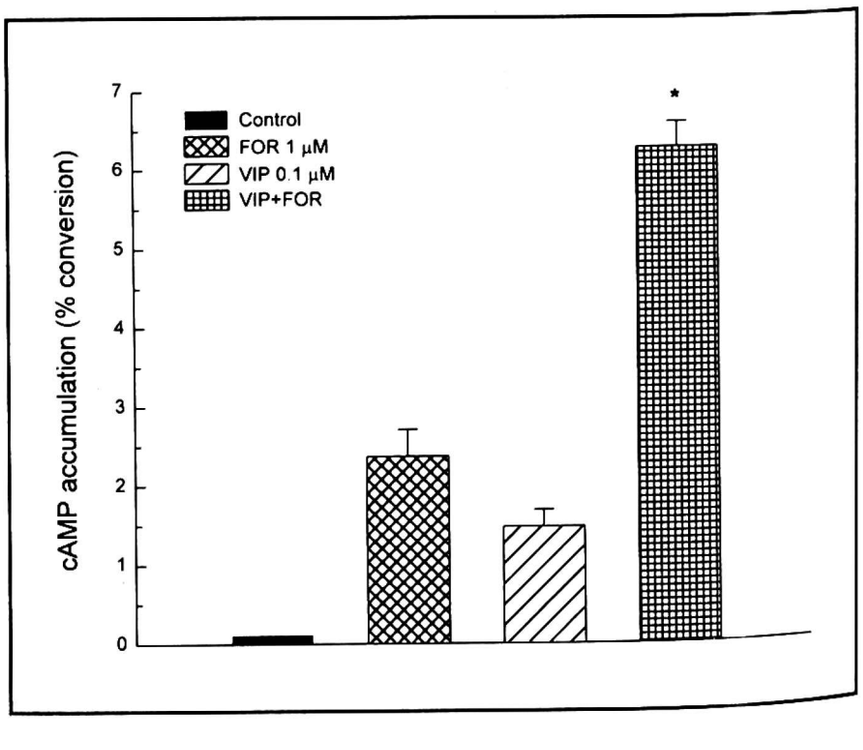

Fig. 3. The effects of vasoactive intestinal peptide (VIP), forskolin (FOR), and their combination, on cyclic AMP formation in the chick pineal gland. Results are means ± SEM from $n = 6$-14 assays/group. *, $p < 0.05$ vs. VIP or FOR.

Table I. Effect of VIP on histamine-, isoprenaline-, and forskolin-evoked stimulations of cyclic AMP formation in cerebral cortical slices of chick.

Drug	Concentration (μM)	cyclic AMP (% conversion)	% control	
Control		0.88 ± 0.06(6)	100	
Histamine	1	1.92 ± 0.18(8)	218	
+ VIP	0.01	1.67 ± 0.18(8)	190	NS vs. Histamine
+ VIP	1	2.33 ± 0.25(7)	265	NS vs. Histamine
Control		1.32 ± 0.10(12)	100	
Histamine	100	4.79 ± 0.16(19)	363	
+ VIP	0.01	4.14 ± 0.18(12)	314	NS vs. Histamine
+ VIP	1	3.98 ± 0.28(15)	302	NS vs. Histamine
Control		1.29 ± 0.21(5)	100	
Isoprenaline	10	5.49 ± 0.82(8)	426	
+ VIP	0.01	6.34 ± 0.83(7)	491	NS vs. Isoprenaline
+ VIP	0.1	6.58 ± 0.77(8)	510	NS vs. Isoprenaline
+ VIP	1	6.92 ± 0.94(8)	536	NS vs. Isoprenaline
Control		1.32 ± 0.52(6)	100	
Forskolin	1	5.17 ± 0.40(7)	392	
+ VIP	0.01	5.86 ± 0.33(6)	444	NS vs. Forskolin
+ VIP	1	5.81 ± 0.45(7)	440	NS vs. Forskolin

Data are means ± SEM from the number of experiments given in parentheses. VIP, at indicated concentrations, did not affect basal (control) cyclic AMP synthesis (see also *Fig. 1*).

In agreement with earlier data (5, 6), histamine (1—1000 μM) strongly stimulated the chick pineal cyclic AMP generating system (with an EC_{50} value of approximately 10 μM), reaching a maximal response at 100 μM concentration, i.e. stimulations — in different experiments — between 700—900% of the control value (not shown). A combination of histamine with forskolin produced a synergistic interaction in the chick pineal gland (*Fig. 4*) (see (8) for comparative data concerning some other avian species).

Combination of VIP and histamine, used at concentrations evoking moderate increases in the chick pineal cyclic AMP synthesis, i.e. 0.1 μM and 1 μM, respectively, showed clearly more than additive response; this effect was totally antagonized by a histamine receptor blocker aminopotentidine (1—3 μM; *Fig. 5*).

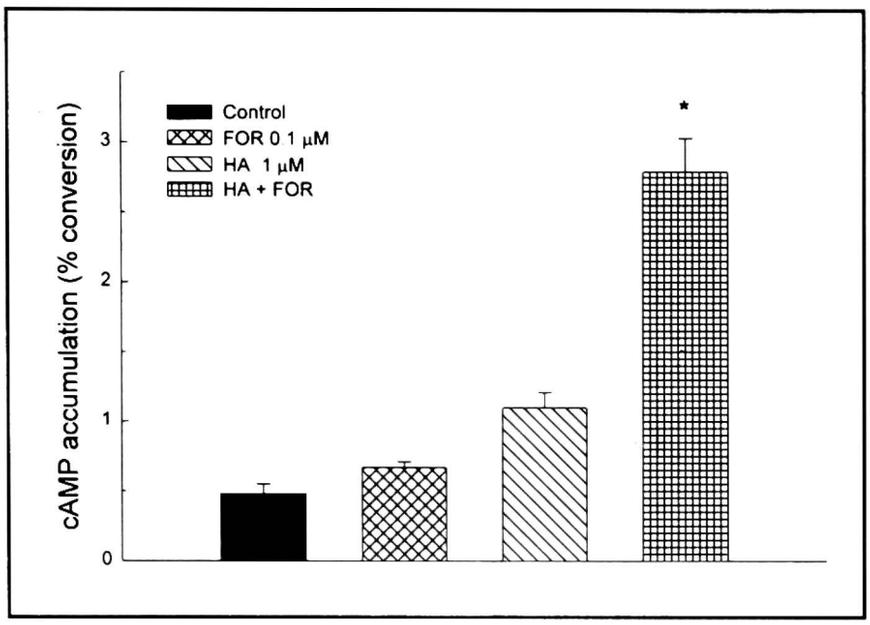

Fig. 4. The effects of histamine (HA), forskolin (FOR), and their combination, on cyclic AMP formation in the chick pineal gland. Results are means ± SEM from n = 6-7 assays/group. *, $p < 0.05$ vs. HA or FOR.

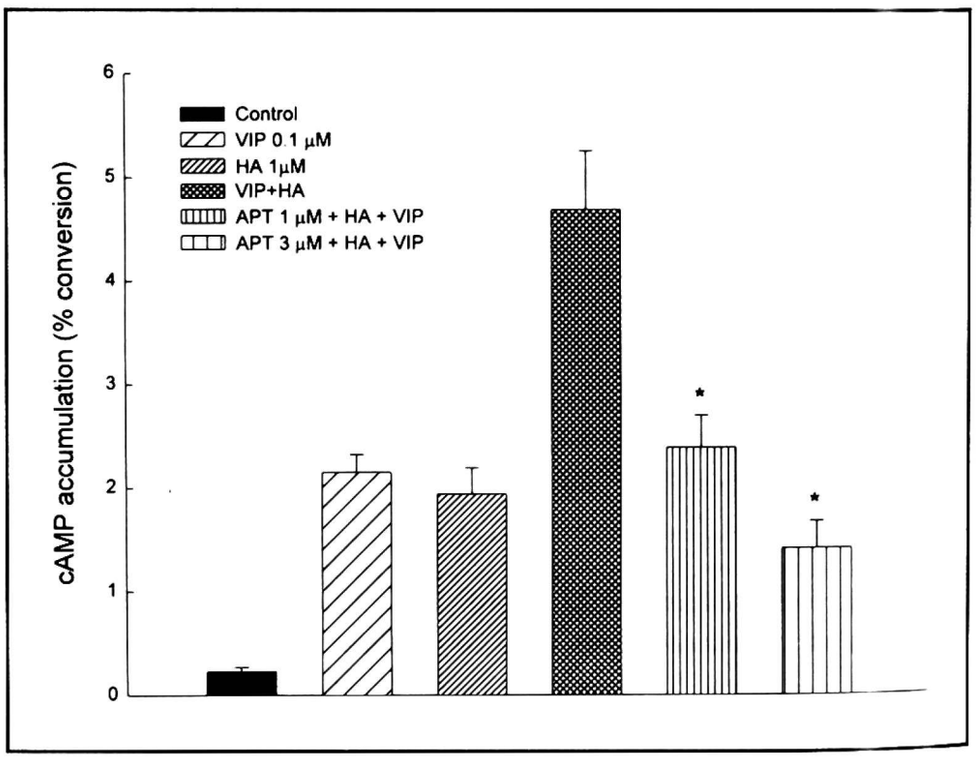

Fig. 5. The antagonism by aminopotentidine (APT) of the effect of a combination of vasoactive intestinal peptide (VIP) and histamine (HA) on cyclic AMP formation in the chick pineal gland. Results are means ± SEM from n = 7—22 assays/group. *, $p < 0.05$ vs. VIP + HA.

DISCUSSION

Histamine and VIP are known stimulators of cyclic AMP synthesis is the CNS of several vertebrate species (18—20). Yet, the two substances show a tissue/species-dependent variability in their action, a phenomenon resulting most probably from an uneven distribution (and density) of appropriate receptors (e.g., 21—23). The present data dealing with VIP give an example of a tissue-specific activity, as in chicks the peptide stimulated cyclic AMP formation potently in the pineal gland, moderately in the retina, and showed no action in the cerebral cortex. In the latter tissue, VIP also did not display any activity when combined with known stimulators of cyclic AMP production

in the chick cerebrum, such as forskolin, a β-adrenoceptor agonist isoproterenol, or histamine, which further suggests that cerebral cortex of chick likely lacks VIP receptors coupled to the nucleotide formation. However, the peptide did stimulate cyclic AMP generation in the mammalian brain, as was shown in a parallel study carried out on cerebral cortical slices of rat (K. Kuba and J.Z. Nowak, unpublished observation; see also 21). On the other hand, histamine effectively activated cyclic AMP generation in the chick pineal gland (5, 6), cerebral cortex (24) and retina (2), but did not affect the nucleotide production in the rat pineal gland (5).

The stimulatory action of VIP and histamine on cyclic AMP formation in the chick pineal gland is interesting in that both substances may be involved in the regulation of the pineal hormone melatonin. In fact, VIP has earlier been shown in chick pineal cell cultures to stimulate the activity of serotonin N-acetyltransferase (NAT; a key regulatory enzyme in melatonin biosynthesis; 25, 26) and melatonin release (10, 11, 27). In contrast to VIP, the effect of histamine on melatonin release from organ-cultured chick pineals was comparatively weak (but significant; 9), and non-significant on NAT activity (unpublished data; see also 9). Such a difference in the action between VIP and histamine in the chick pineal gland seems unexpected because both neuromodulators strongly stimulate cyclic AMP production in this tissue, and — importantly — cyclic AMP is a crucial factor for the process of NAT induction (25, 28). It is possible, however, that VIP and histamine influence the cyclic AMP generating systems localized to different pineal cell populations, i.e. melatonin-synthesizing cells (VIP-sensitive) and other pineal cells not involved in the hormone production (histamine-sensitive). The ability of forskolin, a direct activator of adenylyl cyclase (29), to strongly potentiate the histamine- and VIP-evoked cyclic AMP responses in the chick pineal suggests a likely receptor-type mode of action for the two neuromodulators.

The present data showed that VIP and histamine, when combined together, can interact in a synergistic manner to stimulate cyclic AMP production in the chick pineal. Furthermore, the VIP-histamine synergism was abolished by aminopotentidine, an antagonist of the (mammalian) H_2-type histamine receptor (4), suggesting that the interaction is specific and dependent on a signal deriving from the activation of H_2-like receptor, or as suggested earlier based on atypical pharmacology of the histamine-evoked cyclic AMP response in chicks and ducks (5—7) an avian-specific H_2-like receptor (H_{AV}). The precise mechanism underlying such a synergistic interaction between VIP and histamine in the chick pineal gland remains unknown, and at least two possibilities can be offered, e.g., an interaction between histamine- and VIP-receptors both being coupled to adenylyl cyclase, or those linked to different effectors, such as adenylyl cyclase and phospholipase C (PLC). Recent evidence shows that in the chick pineal gland, histamine, in addition to

eleveting cyclic AMP production, is capable of increasing — in aminopotentidine-sensitive manner — both levels of IP_3 (whole glands; 30) and $[Ca^{2+}]_i$ (in acutely dissociated pineal cells; J.B. Zawilska and P.M. Iuvone, unpublished observation), which may suggest a linkage of H_{AV} with two effector systems, i.e. adenylyl cyclase and PLC. An observation supporting such a possibility has recently been reported for the mammalian H_2-type receptor (31, 32). On the other hand, there are also reports showing a linkage of a VIP-ergic signal, in addition to adenylyl cyclase/cyclic AMP pathway, with intracellular Ca^{2+} mobilization (33, 34), which makes the mechanism responsible for the observed VIP-histamine interaction in the avian pineal gland even more complex.

Whatever the mechanism underlying a synergistic histamine-VIP interaction with regard to cyclic AMP synthesis in the chick pineal gland, the present data show that both neuromodulators can positively cooperate to induce a significant biological effect. Keeping this in mind, it would be highly interesting to study the effect of a combination of VIP and histamine on melatonin biosynthesis in (or release from) the avian pineal gland.

CONCLUSIONS

In chicks,

1. Histamine stimulated cyclic AMP synthesis in three tested tissues showing the following order of sensitivity to the amine: pineal gland > cerebral cortex > retina.

2. VIP stimulated cyclic AMP formation strongly in the pineal gland, moderately in retina, and had no action in cerebral cortex.

3. Combination of histamine or VIP with forskolin resulted in a synergistic response in the pineal gland; a similar, although weaker, synergistic interaction was observed between histamine and forskolin in cerebral cortex, whereas in this tissue VIP did not affect the effects produced by histamine, isoprenaline, or forskolin.

4. The synergistic interaction between histamine and VIP on cyclic AMP formation in the pineal gland was antagonized by aminopotentidine, an H_2-histamine receptor blocker.

5. The observation that histamine and VIP can positively cooperate to induce a significant biological response in the chick pineal gland renders both substances to a group of functionally significant regulators of the pineal physiology in avians.

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