The retina and the pineal organ share a number of similarities. Both tissues contain melatonin (Baker & Quay, 1969), the content of which shows a circadian rhythm (Wurtman et al., 1980). The rhythms of the two organs seem to be independent of each other (Hamm & Menaker, 1980). Since a day-night rhythm for serotonin and catecholamine concentrations has been shown to occur in the pineal (see Wurtman et al., 1968; Wolstenholme & Knight, 1971), we decided to see whether a similar phenomenon existed in the retina. Previous reports have established that dopamine is a likely neurotransmitter in the retina (Kramer, 1976), but the evidence for serotonin having such a role was controversial (Ehlinger & Florén, 1980; Osborne, 1980; Osborne & Richardson, 1980). However, our recent studies on the frog retina have gone far to establish that in this species, at least, serotonin

**Fig. 1. The activation of cyclic nucleotide phosphodiesterase by fraction Sb and calmodulin**

Phosphodiesterase activity was measured in the presence of various amounts of calmodulin (\(\Delta\)) and synaptosomal cytosol factor (\(\Delta\)) under standard assay conditions, which included 40 mM-Tris/HCl (pH 7.4), 0.1 mM-MnCl, 2 mM-cyclic AMP and 0.1 mM-CaCl,. Reactions were carried out at 37°C. Ca²⁺ was excluded from a series of assays including fraction Sb (\(\bullet\)).

![Graph](image)

The present investigation, we report the activation of brain phosphodiesterase by a Ca²⁺-independent component in the synaptosomal soluble fraction.

An enriched synaptosomal preparation was isolated from sheep brain cortex by a modified method of Mena et al. (1980). The synaptosomal fraction collected from the Ficoll gradient stage was washed with iso-osmotic sucrose and fractionated on a discontinuous gradient containing 0.8 M-1.1 M-1.6 M-sucrose.

Two prominent bands were collected and, on transferring the material from the 1.1 M-1.6 M-sucrose interface to 0.32 M-sucrose, lysis (30%) of the synaptosomes occurred as indicated by the release of occluded lactate dehydrogenase (EC 1.1.1.27). Little enzymic activity (<5%) was present in synaptosomes collected from the 0.8 M-1.1 M-sucrose layer. After osmotic shock the synaptosomal soluble fraction was collected by centrifugation at 100000 g. The soluble components were now heat-treated (100°C for 10 min) and the supernatant was collected, dialysed and passed through a DE-52 column (Whatman) that had been equilibrated in 50 mM-sodium phosphate (pH 7.4). The column washings were collected (fraction Sb). Calmodulin and phosphodiesterase (EC 3.1.4.1) were pre-

**Serotonin and dopamine fluctuations in the frog retina in response to dark and light adaptations**

THOMAS NESSELHUT* and NEVILLE N. OSBORNE

The retina and the pineal organ share a number of similarities. Both tissues contain melatonin (Baker & Quay, 1969), the content of which shows a circadian rhythm (Wurtman et al., 1968; Hamm & Menaker, 1980). The rhythms of the two organs seem to be independent of each other (Hamm & Menaker, 1980). Since a day-night rhythm for serotonin and catecholamine concentrations has been shown to occur in the pineal (see Wurtman et al., 1968; Wolstenholme & Knight, 1971), we decided to see whether a similar phenomenon existed in the retina. Previous reports have established that dopamine is a likely neurotransmitter in the retina (Kramer, 1976), but the evidence for serotonin having such a role was controversial (Ehlinger & Florén, 1980; Osborne, 1980; Osborne & Richardson, 1980). However, our recent studies on the frog retina have gone far to establish that in this species, at least, serotonin
is a likely transmitter of certain amacrine cells (Osborne et al., 1981).

Frogs (Rana temporaria) were bought from a local supplier and kept in continuous dark or light for a period of 36 h. The light source was 60 W light bulbs. The retinas were then dissected in dim light, and an HClO4 extract was prepared, which was directly analyzed by high-pressure liquid chromatography (see Fig. 1). We have analyzed the retinas from a number of light- and dark-adapted animals and established beyond doubt that more dopamine (four times the amount) exists in the light-adapted retinas than in the dark-adapted tissues. In contrast the serotonin concentration in the light-adapted retinas is less (half the amount) than in the dark-adapted (see Fig. 1). A clear fluctuation was also observed for an unidentified substance (see Fig. 1). With the procedure used, the dopamine and serotonin metabolites are simultaneously measured and while we have an indication that they fluctuate with light and dark adaptations, we have not yet been able to establish this unequivocally.

We have also used a monoclonal antibody specific for serotonin (in conjunction with Dr. A. C. Cuello) to study the distribution of cell bodies containing the amine in light- and dark-adapted retinas. Examination of more than 200 fields of view with a ×25 objective showed that the average amount of serotonin cell bodies per field in dark-adapted retinas was 2.07 ± 0.3 (n = 105), whereas in the light-adapted retinas it was 0.56 ± 0.2 (n = 106).

The present experiments establish beyond doubt that in the frog retina there is a fluctuation of dopamine and serotonin content depending on exposure to light or dark. The fluctuation of biogenic amines also occurs in the pineal, where the noradrenaline content is high at night and low by day, whereas the inverse is true for serotonin (see Wurtman et al., 1968; Wolstenholme & Knight, 1971). However, in the retina the serotonin and dopamine are situated in neurones, namely the amacrine cells, whereas in the pineal this does not seem to be the case. The similarity between the retina and pineal in its biochemistry and rhythm is fascinating and requires intensive study.

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**Mechanism of neurotensin inactivation by subcellular fractions of rat brain**

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Neurotensin was first isolated from the bovine hypothalamus and shown to be a tridecapeptide with the structure <Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Pro-Tyr-Ile-Leu*> (Carraway & Leeman, 1975). It was subsequently shown that neurotensin has widespread distribution in the central nervous system and gastrointestinal tract. Although a number of biological effects have been ascribed to neurotensin (see Fernstrom et al., 1980, for review), its localization and release from synaptosomes and the presence of receptors in synaptic membranes suggest that this peptide may have a role in neurotransmission.

It is known that neurotensin loses its immunoreactivity on incubation with extracts of rat brain and hypothalamus (Dupont & Merand, 1978) and also that the peptide is degraded by a cation-sensitive neutral endopeptidase from bovine pituitary (Wilk & Orlowski, 1980), but little is known of the mechanism by which the degradation takes place. In the present study, the products of neurotensin degradation formed on incubation of the peptide with particulate and soluble fractions of rat cortex, hypothalamus, thalamus and pituitary have been isolated by h.p.l.c. and identified by amino acid analysis.

Dialysed fractions (equivalent to two hypothalami) were prepared as described previously (Griffiths et al., 1980) and incubated with 61 nmol of neurotensin in 1 ml total volume (0.1 M-sodium phosphate, pH 7.38) at 37°C for 5, 15, 30 and 60 min. Peptides were extracted from the freeze-dried incubation mixture using 13 mM-trifluoroacetic acid in 80% metha-