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UPTAKE, LOCALIZATION AND RELEASE OF SEROTONIN IN THE CHICK RETINA

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SUMMARY

1. High pressure liquid chromatography and electrochemical detection have shown that serotonin exists in the chick retina (127 ng/g wet weight). No other indoleamine was identified.

2. Immunofluorescent histological studies showed that the endogenous serotonin was localized apparently in those cell bodies and processes which took up exogenous [^{3}H]serotonin as revealed by autoradiography. These serotonergic neurones can be destroyed by injecting kainic acid into the eye.

3. Isolated chick retina accumulated exogenous [^{3}H]serotonin. Kinetic analysis revealed the presence of two saturable uptake systems: a 'high affinity' mechanism with an apparent K_m of $5{\cdot}9 \times 10^{-8}$ M and a V_{max} of $0{\cdot}143 \times 10^{-13}$ mol/mg wet weight . min and a low affinity mechanism with an apparent K_{m_2} of $1{\cdot}8 \times 10^{-3}$ M and V_{max} of $0{\cdot}12 \times 10^{-9}$ mol/mg wet weight . min.

4. The uptake of serotonin was temperature-sensitive and sodium-dependent and Lilly 110140 and chlorimipramine were potent inhibitors of the amine uptake.

5. Autoradiographic studies indicated that neuronal processes associated with the innermost and outermost areas of the inner plexiform layer and perikarya situated in the inner nuclear layer are the sites which accumulated exogenous [^{3}H]serotonin.

6. [^{3}H]Serotonin accumulated in the retina was released by increasing the external K^+ concentration. This release was Ca^{2+}-dependent. Additionally, autoradiographic studies show that [^{3}H]serotonin taken up by the serotonin neurones was also released by Ca^{2+}-dependent K^+ depolarization of the retina.

INTRODUCTION

The retina is an excellent system for studying the morphology, physiology and biochemistry of specific neuronal systems. Several compounds such as acetylcholine, dopamine and GABA are considered to be neurotransmitters in the retina (Graham, 1974; Kramer, 1976; Neal, 1976; Bonting, 1976), as they are in the brain (see Iversen, 1978). In recent years serotonin has been proposed as having a transmitter role in the retina (see Osborne, 1982), although earlier studies by Ehinger and co-workers (see Ehinger & Florén, 1980; Florén & Hansson, 1980) resulted in the suggestion that an indole compound closely related to serotonin was the true retinal transmitter. This

arose from the observation that specific neurones in the retina accumulated exogenous serotonin (Ehinger & Florén, 1978), yet all attempts to localize serotonin-containing neurones failed. Recent immunohistochemical studies have, however, shown the presence of serotonin-containing neurones in retinas of a variety of species (Osborne, 1982; Osborne, Nesselhut, Nicholas & Cuello, 1981; Osborne, Nesselhut, Nicholas, Patel & Cuello 1982*a*), although the level of serotonin in mammalian retinas is too low for localization. No evidence could be found for the presence of any indoleamine other than serotonin in any of the retinas examined (Osborne *et al.* 1982*a*).

In this study we provide evidence that serotonin is a transmitter in the chick retina where, it is shown, defined serotonergic neurones specifically take up exogenous serotonin and release it on stimulation.

METHODS

2–9 d old chicks were used in all experiments. Birds were kept under standard laboratory conditions at 28 °C with food and water *ad libitum*. The birds were always killed between 10.00 a.m. and 12.00 a.m. in order to reduce the effect of daily rhythms on retinal serotonin content (Baker & Hoff, 1979).

Uptake experiments. Pieces of retina, each weighing 2 mg (S.E. of mean $\pm 0{\cdot}1$ mg, $n = 15$) were 'punched out' and placed in a beaker of cold Krebs–bicarbonate medium which consisted of (mM): NaCl, 114; KCl, 4·57; $CaCl_2$, 2·44; KH_2PO_4, 1·14; $MgSO_4.7H_2O$, 1·14; ascorbate, 0·1; and pargyline, 0·1. The pH of the solution was maintained at 7·3, by constant bubbling with a mixture of 95% O_2–5% CO_2. Two pieces of retina were then placed in a vial containing 2 ml of medium. After pre-incubation for 10 min at 37 °C in a shaking water bath, varying amounts of [^{3}H]serotonin (The Radiochemical Centre, Amersham, 16 Ci/mmol) were added to the incubation medium and the incubation was continued for various periods of time. When it was completed, the pieces of retina were recovered with forceps and rapidly rinsed in 20 ml ice-cold medium. It had been previously established that no significant release of radioactivity from the tissue occurred during the washing period. Pairs of retinal pieces were placed in vials containing 0·5 ml tissue solubilizer (Soluene-350, Packard) for at least 2 h at room temperature before adding 10 ml Dimilume (Packard). Radioactivity was measured in a liquid scintillation spectrometer. A small amount of radioactive incubation solution (100 μl) was dissolved in 10 ml Dimilume and also counted. The counting efficiency of ^{3}H varied between 30 and 35%.

The amount of [^{3}H]serotonin accumulated by the tissues was calculated by taking the difference of values obtained at 0 and 37 °C (Shaskan & Snyder, 1970). Unlabelled serotonin was added to the labelled compounds to produce the solutions containing higher concentrations of serotonin required for kinetic studies. Kinetic constants were determined by computer, where an iterative method was used to fit data directly to rate equations for a single or two carrier model (Osborne, 1980).

Release experiments. Isolated whole retinas were incubated in medium containing [^{3}H]serotonin (0·1 μM) for 20 min at 37 °C. The retinas were recovered, rinsed thoroughly and each was transferred to a 1 ml chamber containing fresh medium. The tissue was then superfused at a rate of 0·5 ml/min. Fractions (1 ml) of the superfusate were collected, and after the addition of 10 ml Quickzint 294 scintillation solution (Koch Light Laboratories), the radioactivity in each fraction was measured. Preliminary chromatographic experiments established that approximately 94% of the radioactivity recovered from the tissue and also that released into the superfusates was unmetabolized [^{3}H]serotonin.

Assay of serotonin. High pressure liquid chromatography (h.p.l.c.) with electrochemical detection was used to determine the serotonin content of the chick retina. The apparatus was equipped with a C-18 reverse phase column and an LC-4 amperometric detector. The electrochemical detector was set at 2 nA/V with a potential of 0·8 V. The mobile phase was 0·02 M-sodium citrate buffer pH 3·7 containing 0·2 mM-octane-1-sulphonic acid and 6·5% methanol. Dihydroxybenzylamine (DBZ) was routinely added to the perchloric acid extract. In the system used, serotonin was clearly separated from a number of other indoleamines (see Osborne *et al.* 1982*a*).

Autoradiography of serotonin. Freshly dissected retinas were placed in a Krebs–bicarbonate medium containing ascorbate (0·1 mM) and pargyline (0·1 mM) as described above. The pH of the medium was kept at 7·3 by constantly bubbling with a mixture of 95% O_2–5% CO_2. After a pre-incubation for 10 min at 37 °C, [^{3}H]serotonin (0·1–1 μm) was added and the incubation continued for 10–20 min. In some experiments Lilly 110140 (0·1 mM) or desimipramine (0·1 mM) was added to the incubation medium. Tissues were usually fixed in 5% cold glutaraldehyde in 0·15 M-cacodylate buffer (pH 7·4) for 2–3 h after the incubation phase. However, in some experiments the tissues were first placed for 5 min before fixation in 5 ml of medium modified by the addition of high KCl (25 mM) or high KCl minus Ca^{2+}. After rinsing for 30 min, tissues were post-fixed in 1% osmium tetroxide for 2 h at room temperature. The tissues were then dehydrated in ethanol and propylene oxide, re-embedded in Epon 812 and 2 μm sections cut and subjected to autoradiography using Ilford L4 Nuclear Track Emulsion.

Immunohistochemistry. Retinas were immersed in freshly prepared 4% paraformaldehyde in 0·1 M-phosphate buffer pH 7·2 for 1–3 h and kept in the same buffer containing 30% sucrose, for periods of several hours up to 3 d, 10 μm frozen sections were obtained at −20 °C and recovered on gelatine-coated glass slides. The sections were then incubated overnight at 4 °C with rat × rat antibody (YC 5/45 HL). The monoclonal antibody was derived from a rat × rat hybrid myeloma and was shown to recognize serotonin in formaldehyde tissue sections (Cuello & Milstein, 1981; Consolazione, Milstein, Wright & Cuello, 1981). The rat antibody was developed by an anti-rat IgC FiTC-conjugated immunoglobin (Miles, U.K.). The monoclonal antibodies and development fluorescent antibodies were diluted 1:200 and 1:20 respectively in phosphate buffered saline (PBS) containing 0·2% Triton X-100. The glycerine PBS mounted sections were observed with a microscope equipped for epifluorescence optics. Photographs were taken with Kodak Tri-X Film (400 ASA). The positive immuno-reaction was obliterated when the monoclonal antibody YC5/45 HL was pre-absorbed with an excess of serotonin (1 mM).

Kainic acid lesions. Lesions were produced by injecting 10 μl of 6 mM-kainic acid, brought to pH 7 with NaOH, into the right eye of 2 d old chicks, using a Hamilton syringe with a 26 gauge needle. Control birds received 10 μl of distilled water or colchicine (0·5 μg), brought to pH 7 with NaOH. Two weeks later the animals were killed and their retinas analysed.

RESULTS

Occurrence of serotonin and effect of kainic acid. The h.p.l.c. and electrochemical procedure used allows the complete separation of serotonin from the following possible substances which may occur in the retina: dopamine, dihydroxyphenylalanine, 5-hydroxytryptophan, 5,7-dihydroxytryptamine, 5,6-dihydroxytryptamine, 3-methoxytryptamine and bufotenine (see Osborne *et al.* 1982*a*). Fig. 1 shows the h.p.l.c. separation of a crude perchloric acid extract of chick retina. In the lower trace, an internal standard of serotonin was added to the extracts. On this basis serotonin was identified in the retina and the concentration determined as 127 ± 15 ng/g fresh weight of tissue (see Table 1).

The effect of prior injection of kainic acid and colchicine on the levels of serotonin in the chick retina as determined by h.p.l.c. is shown in Table 1. Kainic acid caused a drastic decrease in the serotonin level while colchicine had no obvious effect. It is known that in the chick retina kainic acid treatment selectively destroys the amacrine cells and horizontal cells, whereas colchicine has a selective effect on the ganglion cells (Buckerfield, Oliver, Chubb & Morgan, 1981).

Localization of serotonin. By using a monoclonal antibody specific for serotonin (Cuello & Milstein, 1981; Consolazione *et al.* 1981), immunoreactive sites were observed only in some amacrine cell bodies situated in the inner nuclear layer and in processes in the inner plexiform layer (Pl. 1). No immunoreactive somata were

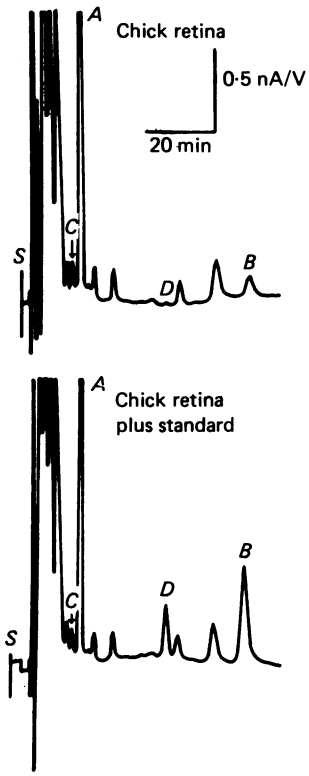

Fig. 1. The upper trace shows an h.p.l.c. separation of various substances in a perchloric acid extract of chick retina. The electrochemical detector was set at 2 nA/V with a potential of 0·8 V and separation was achieved with a C-18 reverse phase column using 0·2 M-sodium citrate buffer pH 3·7 containing 0·2 mM-octane-1-sulphonic acid and 6·5% methanol. Dihydroxybenzylamine was routinely added to the perchloric acid extract as an internal standard. *S*, start. Peaks corresponding to 5-hydroxytryptophan (*C*), dopamine (*A*), 5-hydroxyindoleacetic acid (*D*) and serotonin (*B*) were identified in the frog retina extract (upper trace). Because of the position of the peak corresponding to *C*, it can only be tentatively identified as 5-hydroxytryptophan. Peaks corresponding to serotonin and 5-hydroxyindoleacetic acid were confirmed by adding internal standards of these substances to the extract as shown in the lower trace.

TABLE 1. Effect of kainic acid and colchicine on the serotonin content of the chick retina. Each experimental value is the mean ± S.E. for eight determinations

	Serotonin content (ng/g wet weight)
Normal retina	127 ± 15
Control retina (eye injected with water)	120 ± 19
Kainic acid-treated retina	28 ± 9
Colchicine-treated retina	130 ± 36

observed in the outer nuclear layer or the ganglion cell layer, nor was immunoreactivity associated with the outer plexiform layer or Müller cells. Immunoreactive amacrine cells have a single process which descends directly into the inner plexiform layer where it ramifies as two bands situated on the innermost and outermost areas of the inner plexiform layer. Tangential sections taken through the inner nuclear layer showed that serotonin perikarya were distributed throughout the inner nuclear layer (Pl. 1).

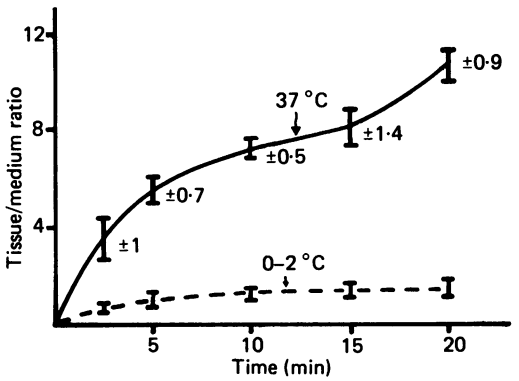

Fig. 2. Time courses of [^{3}H]serotonin uptake into chick retina incubated in physiological solution containing 0·1 μM-[^{3}H]serotonin. Vertical bars indicate mean ± S.E. of mean where n is at least 5.

One week after the kainic acid lesion the prominent cell bodies in the inner nuclear layer and processes in the inner plexiform layer which normally contain serotonin were not to be seen. The total amount of perikarya in the inner nuclear layer was greatly reduced after kainic acid treatment, although the area could still be discerned. The rest of the retina appeared normal in structure.

Autoradiographic studies. The autoradiographical studies supported many of the other data reported. First, radioactive grains were found in somata situated in the inner nuclear layer and in two layers situated in the innermost and outermost areas of the inner plexiform layer (Pl. 2*A*). Thus the distribution of neurones in the chick retina taking up exogenous [^{3}H]serotonin closely parallels the localization of serotonin in the retina. Secondly, retinas previously incubated in [^{3}H]serotonin and then exposed to high potassium (25 mM) showed an almost complete lack of grains in the autoradiographical pictures (Pl. 2*B*). These results suggest that the [^{3}H]serotonin taken up and released into the medium by KCl originates from serotonin-accumulating neurones. The effect of high K was nullified when Ca^{2+} was eliminated from the physiological medium (picture not shown). Thirdly, retinas incubated in [^{3}H]serotonin in the presence of 10^{-4} M-Lilly 110140 revealed no sites which had specifically accumulated radioactivity (Pl. 2*C*). The Lilly 110140 clearly interferes with the influx of [^{3}H]serotonin into the specific population of amacrine neurones which would normally accumulate the exogenous amine. Desimipramine at 10^{-4} M also had a significant effect on the uptake of [^{3}H]serotonin as revealed by autoradiography, although some grains could be seen in amacrine cell bodies (picture not shown).

Kinetics of [^{3}H]serotonin uptake. When chick retinas were incubated with

physiological bicarbonate solution containing [^{3}H]serotonin (0·1 μm), there was a fairly linear accumulation of radioactivity into the retinas for the first 10 min, reaching a tissue/medium ratio of about 7 at this time (Fig. 2). Because of the large volume of medium used (2 ml) compared with the small weight of tissue (about 4 mg), the concentration of [^{3}H]serotonin in the medium after 10 min incubation at 37 °C fell by less than 4 %. At 0 °C no active uptake of serotonin should take place, which explains the low tissue–medium ratios (see Fig. 2). Accordingly, the kinetic

TABLE 2. [^{3}H]serotonin uptake by chick retina. Two pieces (approximately 2 mg each) were incubated at 0 and 37 °C for 10 min and then incubated for a further 10 min with radioactive serotonin. The influx of serotonin was then calculated (observed values) by subtracting the values at 0 °C from those at 37 °C. Each experimentally determined value is the mean ± S.E. of mean for five determinations. The predicted values are those for a two-carrier system with the following kinetic parameters: $K_{m_1} = 5{\cdot}9 \times 10^{-8}$ M, $V_{max_1} = 0{\cdot}143 \times 10^{-13}$ mol/mg.min; $K_{m_2} = 1{\cdot}8 \times 10^{-3}$ M, $V_{max_2} = 0{\cdot}12 \times 10^{-9}$ mol/mg.min; S.D. = 5·7 %

Exogenous [^{3}H]serotonin (μM)	Serotonin accumulated by retina (pmol/mg.10 min)			
	Observed	Predicted V_{max_1}	Predicted V_{max_2}	Predicted V_{max_1} & V_{max_2}
0·05	10·1 ± 0·7	6·5	3·5	10·0
0·075	14·2 ± 0·9	7·9	5·2	13·2
0·1	16·8 ± 0·6	8·9	7·0	16·0
0·2	24·3 ± 1·0	11·0	14·0	25·0
0·5	46·5 ± 1·1	12·7	35·1	47·9
0·75	65·4 ± 1·0	13·2	52·7	65·9
1·0	87·1 ± 2·3	13·5	70·2	83·8
2·5	196·0 ± 9·0	13·9	175·5	189·5
5·0	362·3 ± 14·8	14·1	350·6	364·8
10·0	775·2 ± 29·2	14·2	699·9	713·6

studies of serotonin uptake into the retinas using 10^{-5}–5×10^{-8} M-exogenous [^{3}H]serotonin were all performed at an incubation time of 10 min at both 0 and 37 °C. The results were analysed by an iterative method where the corrected data, i.e. uptake at 37 °C minus accumulation at 0 °C were fitted directly into rate equations with a digital computer (Table 2). Models consisting of a single- or two-carrier system were examined. Assessed in terms of nearness to fit of data to the iterative model parameters, a two carrier model was preferable (see Table 2) and gave a standard deviation of 5·7 %. The data fitted into a single-carrier produced a deviation of 42 %. The predicted values derived from the two-carrier model are listed in Table 2. The observed values produced the following kinetic constants: $K_{m_1} = 5{\cdot}9 \times 10^{-8}$ M, $K_{m_2} = 1{\cdot}8 \times 10^{-3}$ M, $V_{max_1} = 0{\cdot}143 \times 10^{-13}$ mol/mg.min, $V_{max_2} = 0{\cdot}12 \times 10^{-9}$ mol/mg.min.

Characteristics of serotonin uptake. The effect of external sodium concentration on the [^{3}H]serotonin (0·1 μM) uptake was examined by varying the external sodium concentrations. Fig. 3 shows that serotonin uptake increases almost linearly with increasing extracellular sodium concentrations, thus showing that the uptake of serotonin is sodium-dependent. Furthermore, there was no effect of 1 mM of either dopamine, noradrenaline or tryptamine on the uptake of 0·1 μM-[^{3}H]serotonin at

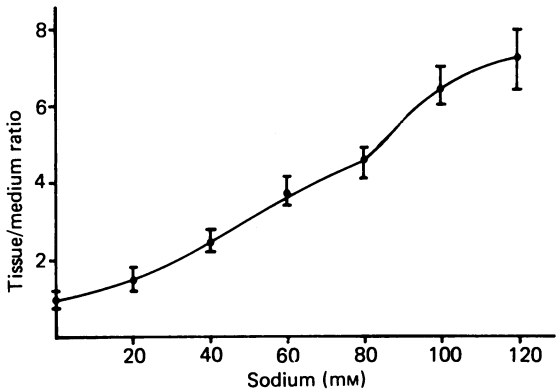

Fig. 3. Effect of sodium concentration on the accumulation of [^{3}H]serotonin by chick retina. Pieces of retina were incubated for 10 min with 0·1 μM-[^{3}H]serotonin in media containing various concentrations of sodium ions. Each point is the mean with $\pm$S.E. of mean of at least four determinations.

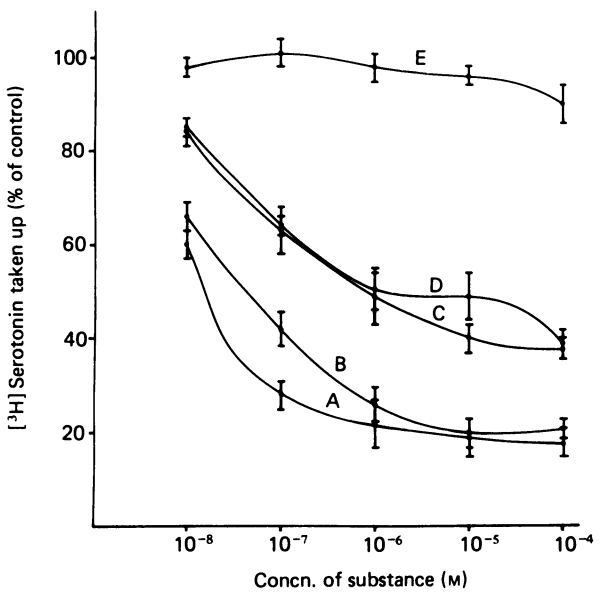

Fig. 4. Effect of various substances on the uptake of serotonin by chick retina. Pieces of retina were incubated for 10 min with 0·1 μM [^{3}H]serotonin and various concentrations of either Lilly 110140 (*A*), chlorimipramine (*B*), benztropine (*C*), desimipramine (*D*) or dinitrophenol (*E*). Each point is the mean with $\pm$S.E. of mean determinations.

different external sodium concentrations. The uptake of 1 mM-[^{3}H]serotonin was, however, slightly influenced by dopamine (12% decrease), noradrenaline (15% decrease) or tryptamine (23% decrease) at 1 mM. These results indicate that the high affinity serotonin uptake process is specific for the indoleamine and can be characterized by studying the uptake of serotonin at low concentrations, i.e. 0·1 μM.

The effect of Lilly 110140, chlorimipramine, dinitrophenol, benztropine and

desimipramine on the uptake of 0·1 μM-[^{3}H]serotonin was also studied. As shown in Fig. 4, the most potent effects were found with Lilly 110140, and chlorimipramine, both known to be potent inhibitors of serotonin uptake.

K^+-stimulated release of [^{3}H]serotonin. The efflux of [^{3}H]serotonin was examined by first incubating retinas in 0·1 μM-[^{3}H]serotonin and then rinsing thoroughly in physiological saline. The tissues were placed in vessels perfused with physiological

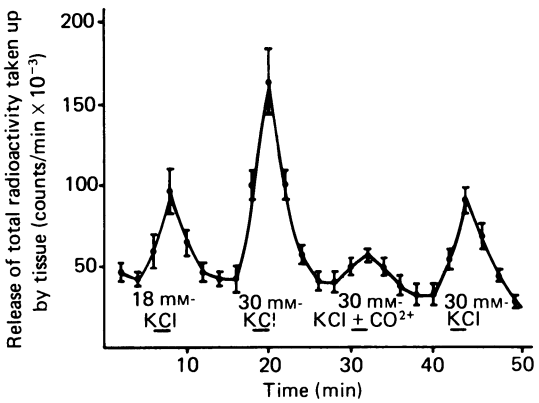

Fig. 5. The efflux of [^{3}H]serotonin from chick retina in normal and K^+-rich physiological solution. Retinas were incubated for 20 min with physiological solution containing 0·1 μM [^{3}H]serotonin, washed thoroughly and transferred individually to 1 ml chambers which were continuously perfused with physiological solution at a rate of 1 ml/2 min. The perfused physiological solution was changed for a modified form of it containing enriched levels of KCl plus 15 mM-Co Cl_2. Each value is the mean ±S.E. of mean of six different experiments.

saline. The radioactivity in 1 ml fractions of the effluent was constantly monitored. A steady efflux of radioactivity (about 2% of total radioactivity taken up was spontaneously released every 2 min) was usually reached in 16 min. When medium containing a high concentration of KCl (between 18 and 30 mM) was perfused through the chamber for a short period it stimulated the efflux of [^{3}H]serotonin. In a 2 min stimulation by 30 mM-KCl about 13% of total radioactivity taken up was released. The efflux rates returned to basal levels when the high KCl physiological saline was replaced by normal physiological solution (see Fig. 5). The K^+-stimulated increase in [^{3}H]serotonin release was inhibited by the presence of 15 mM-Co^{2+} in the K^+-rich physiological solution. Upon removal of Co^{2+} from the medium, the K^+-rich solution again caused a transient increase in [^{3}H]serotonin efflux.

In some experiments the effect of various 'transmitter-type' molecules (10^{-4} M) on the efflux of [^{3}H]serotonin was also measured. Only dopamine caused a slight transient efflux of [^{3}H]serotonin. Substance P, cholecystokinin, GABA and noradrenaline were without apparent effect.

DISCUSSION

The results show that there is a population of amacrine cells which contain serotonin in the chick retina. The identification of these amacrine cells is based upon the position of their perikarya (in the middle of the inner nuclear layer), the position of their terminal arborizations (the inner plexiform layer), and their sensitivity to kainic acid. Kainic acid is known to destroy chick amacrine cells selectively when injected into the eye, as was done in this study (Buckerfield *et al.* 1981) and, while the present experiments generally confirm this, it could not be excluded from the morphological pictures that other cells in the inner nuclear layer (horizontal cells) were not affected. The serotonin cells are not interplexiform cells, as no fluorescence was detected in the outer plexiform layer. Thus the present finding substantiates the earlier, less conclusive data on the occurrence of serotonin amacrine cells in the chick retina which were presented by Hauschild & Laties (1973). The serotonin amacrine cells have processes which subdivide into two clear laminations situated on the inner and outermost areas of the inner plexiform layers. This distribution of serotonergic processes is different from that found in other retinas so far examined, i.e. frog, lizard, and goldfish (see Osborne *et al.* 1981; Osborne *et al.* 1982*a*).

The biochemical finding that chick retina can concentrate [^{3}H]serotonin from an external medium by a high affinity system confirms other studies (Suzuki, Noguchi & Yagi, 1978; Osborne, 1980; Ehinger & Florén, 1978). The affinity constants found for the chick retina are slightly different from those determined by Suzuki *et al.* (1978) for chick retina. The slight differences in the constants could be explained by the fact that Suzuki *et al.* (1978) determined them by 'hand-constructed' Lineweaver-Burk plots, always a possible source of error (see Cornish-Bowden, 1976). As with other transport processes high affinity serotonin uptake is strongly dependent on both the temperature and serotonin concentration in the medium. Furthermore, chlorimipramine and Lilly 110140, both potent blockers of serotonin uptake in brain tissue (see Shaskan & Snyder, 1970; Wong, Horng, Bymaster, Houser & Molloy, 1974), have strong inhibitory effects on retinal uptake of the amine. The sites in the retina which take up exogenous serotonin are specific for the amine because neither dopamine, noradrenaline nor tryptamine influence the high affinity uptake mechanism for serotonin. Dopamine (Sarthy & Lam, 1979) and noradrenaline (Osborne, 1981) are known to be taken up by specific neurones in the vertebrate retina, while tryptamine is not (Osborne & Richardson, 1980).

The functional significance of high-affinity uptake is not precisely known, though it is generally assumed to be the mechanism which results in the removal and therefore inactivation of a transmitter released by presynaptic terminals (Iversen, 1977). Neurones which take up exogenous serotonin in the retina would be expected to contain the amine if the substance were to have a transmitter role. The results from autoradiography and immunohistofluorescence clearly support the idea that the neurones which take up exogenous serotonin also contain the amine. Serotonergic amacrine perikarya (see Pl. 1) are situated more towards the middle of the inner nuclear layer. This is precisely the area where some perikarya are found to accumulate [^{3}H]serotonin (Pl. 2). Furthermore, layers in the innermost and outermost areas of the inner plexiform layer contain serotonin as shown by immunohistochemical

studies (Pl. 1), and also take up exogenous amine as demonstrated by autoradiography (Pl. 2).

The autoradiographic and biochemical studies strongly suggest that serotonin-accumulating neurones in the chick retina release [^{3}H]serotonin by K^+ depolarization. This release is Ca-dependent because it is inhibited by the presence of Co^{2+} ions in the incubation medium or by the absence of calcium from the medium. It is thought that Co^{2+} ions interfere with the entry of Ca^{2+} into the synaptic endings which results in a reduction in, or abolition of, transmitter release (Weakly, 1973). The finding that dopamine causes a slight increase in the efflux of [^{3}H]serotonin from chick retina, whereas other transmitter-type molecules like substance P, cholecystokinin, GABA and noradrenaline have no effect, suggests that dopamine cells may have inputs on the serotonergic cells.

In summary, the findings presented in this paper show that a sub-population of amacrine cells in the chick retina contain serotonin and possess a high affinity uptake system for serotonin. The morphology of these neurones is different from that of chick amacrine neurones which accumulate exogenous GABA, noradrenaline, dopamine, glycine and may contain substance P (Osborne *et al.* 1982*b*). The serotonin accumulated by the serotonergic neurones can be released by K depolarization in a Ca-dependent manner. In all, these results make a strong case for serotonin being a neurotransmitter in the chick retina.

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REFERENCES

BAKER, P. C. & HOFF, K. M. (1979). Daily variations in the eye's 5-HT stores. *Experientia*, **35**, 780–781.

BONTING, S. L. (1976). *Transmitters in the Visual Process*. Oxford: Pergamon Press.

BUCKERFIELD, M., OLIVER, J., CHUBB, I. W. & MORGAN, I. (1981). Somatostatin-like immunoreactivity in amacrine cells of the chicken retina. *Neuroscience* **6**, 689–695.

CONSOLAZIONE, A., MILSTEIN, C., WRIGHT, B. & CUELLO, A. C. (1981). Immunocytochemical detection of serotonin with monoclonal antibodies. *J. Histochem. Cytochem.* **29**, 1425–1431.

CORNISH-BOWDEN, A. (1976). *Principles of Enzyme Kinetics*. London: Butterworth.

CUELLO, A. C. & MILSTEIN, C. (1981). Use of internally labelled monoclonal antibodies. In *Physiological Peptides and New Trends in Radioimmunology*, ed. BIZOLLON, CH. A., pp. 293–305. Amsterdam: Elsevier/North Holland Press.

EHINGER, B. & FLORÉN, I. (1978). Quantitation of the uptake of indoleamines and dopamine in the rabbit retina. *Expl Eye Res.* **26**, 1–11.

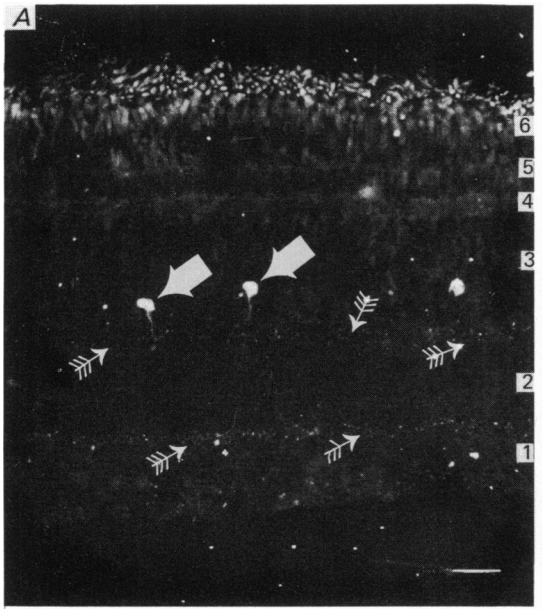
EHINGER, B. & FLORÉN, I. (1980). Retinal indoleamine accumulating neurones. *Neurochem. Int.* **1**, 209–229.

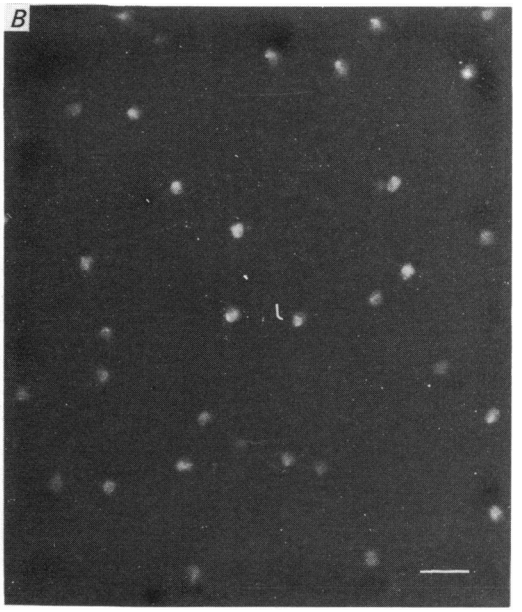
FLORÉN, I. & HANSSON, H. C. (1980). Investigation into whether 5-hydroxytryptamine is a neurotransmitter in the retina of rabbit and chicken. *Invest. Ophthal.* **19**, 117–125.

GRAHAM, L. T. JR. (1974). Comparative aspects of neurotransmitters in the retina. In *The Eye*, vol. 6, ed. DAWSON, H. & GRAHAM, L T. JR., pp. 283–342. New York: Academic Press.

HAUSCHILD, D. & LATIES, A. M. (1973). An indoleamine-containing cell in chick retina. *Invest. Ophthal.* **12**, 537–540.

IVERSEN, L. L. (1977). Inactivation of neurotransmitters. In *Synapses*, ed. COTTRELL, G. A. & USHERWOOD, R. N. R., pp. 137–153. Blackie: Glasgow.





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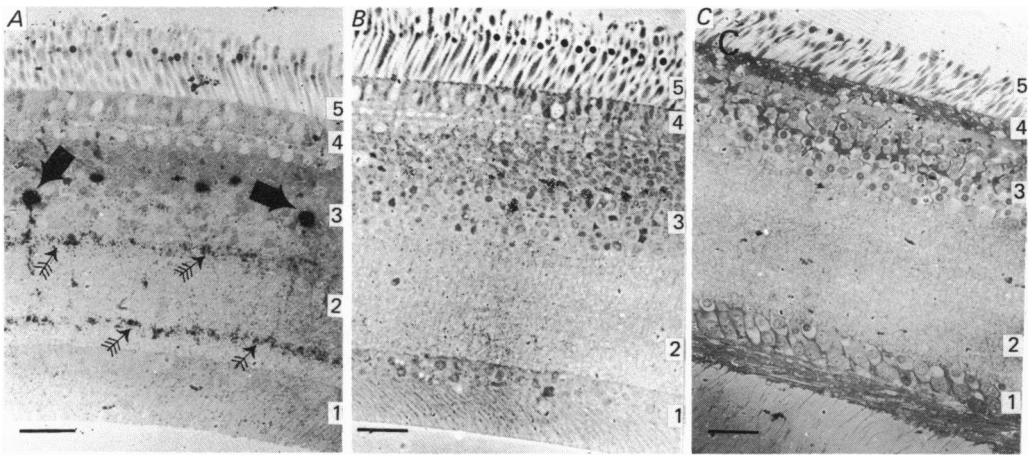
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IVERSEN, L. L. (1978). Chemical messengers in the brain. *Trends in Neuroscience* **1**, 15–16.

KRAMER, S. (1976). Dopamine in retinal neurotransmission. In *Transmitters in the Visual Process*, ed. BONTING, S. L., pp. 165–198. Oxford: Pergamon Press.

NEAL, M. J. 1976). Amino acid transmitter substances in the vertebrate retina. *Gen. Pharmacol.* **7**, 321–332.

OSBORNE, N. N. (1980). *In vitro* experiments on the metabolism, uptake and release of 5-hydroxytryptamine in bovine retina. *Brain Res.* **184**, pp. 283–297.

OSBORNE, N. N. (1981). Noradrenaline, a transmitter candidate in the retina, *J. Neurochem.* **36**, 17–27.

OSBORNE, N. N. (1982). Evidence for serotonin being a neurotransmitter in the retina. In *Biology of Serotonergic transmission*, ed. OSBORNE, N. N., pp. 401–430. Chichester: John Wiley and Sons.

OSBORNE, N. N., NESSELHUT, T., NICHOLAS, D. A. & CUELLO, A. C. (1981). Serotonin: a transmitter candidate in the vertebrate retina. *Neurochem. Int.* **3**, 171–176.

OSBORNE, N. N., NESSELHUT, T., NICHOLAS, D. A., PATEL, S. & CUELLO, A. C. (1982*a*). Serotonin-containing neurones in vertebrate retinas. *J. Neurochem.* (in the Press).

OSBORNE, N. N., NICHOLAS, D. A., DOCKRAY, G. J. & CUELLO, A. C. (1982*b*). Immunoreactivity in retinas of rats, frogs, lizards and chicks. *Expl Eye Res.* **34**, 639–649.

OSBORNE, N. N., & RICHARDSON, G. (1980). Specificity of serotonin uptake by bovine retina: comparison with tryptamine. *Expl Eye Res.* **31**, 31–39.

SARTHY, P. V. & LAM, D. M. K. (1979). The uptake and release of [^{3}H]dopamine in the goldfish retina. *J. Neurochem.* **32**, 1267–1277.

SHASKAN, E. G. & SNYDER, S. H. (1970). Kinetics of serotonin accumulation into slices from rat brain: relationship to catecholamine uptake. *J. Pharmac. exp. Ther.*, **175**, 404–418.

SUZUKI, O., NOGUCHI, F. & YAGI, K. (1978). Uptake of 5-hydroxytryptamine by chick retina. *J. Neurochem.* **30**, 295–296.

WEAKLY, J. N. (1973). The action of cobalt ions on neuromuscular transmission in the frog. *J. Physiol.* **234**, 597–612.

WONG, W. T., HORNG, J. S., BYMASTER, F. P., HOUSER, K. L. & MOLLOY, B. B. (1974). A selective inhibitor of serotonin uptake: Lilly 110140, 3-(p-trifluoro-methylphenoxy)-N-methyl-phenyl-propylamine. *Life Sci, Oxford* **15**, 471–479.

EXPLANATION OF PLATES

PLATE 1

Immunohistochemical demonstration of serotonin in chick retina. The procedure used for localizing serotonin is described in the Methods. *A* is a section through the retina showing the amine to be localized in some perikarya (large arrows) situated in the inner nuclear layer (3) and two layers of terminal situated in the inner nuclear layer (3) and two layers of terminal situated in the inner and outmost areas (small arrows) of the inner plexiform layer (2). No immunoreactivity was associated with the outer nuclear layer (5), outer plexiform layer (4), ganglion cell layer (1) or photoreceptor layer (6). *B* is a section taken through the inner nuclear layer to show the spatial distribution of serotonin perikarya. Scale bar = 50 μm.

PLATE 2

Light microscopy autoradiography of [^{3}H]serotonin uptake and release in the chick retina. Retinas were incubated first in [^{3}H]serotonin alone or [^{3}H]serotonin with 10^{-4} M-Lilly 110140 (see Methods). Retinas incubated in [^{3}H]serotonin were either placed for short periods in fresh physiological solution alone or physiological solution containing high potassium (25 mM) as described in the Methods section. They were then fixed and processed for autoradiography. *A*, retina in normal physiological solution; *B*, retina incubated with K^+-rich physiological solution; *C*, retina in normal physiological solution containing Lilly 110140. Large arrows show serotonin-accumulating perikarya in inner nuclear layer (5) and smaller arrows show serotonin-accumulating terminals in inner plexiform layer (2). 1, ganglion cell layer; 3, outer plexiform layer; 4, outer nuclear layer; 5, photoreceptor layer. Scale bar = 50 μm.