*J. Sci. Soc. Thailand*, 9 (1983) 89-106

# UPTAKE AND RELEASE OF NEUROTRANSMITTER-RELATED COMPOUNDS BY TWO POPULATIONS OF SYNAPTOSOMES ISOLATED FROM RABBIT RETINA

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*(Received 3 December 1982)*

**Abstract**

*Synapses of retina have high affinity uptake mechanisms which may play an important role in the chemical transmission processes of the retina. In this study, a comparison was made among the uptake systems of retinal synaptosomes obtained from the outer plexiform layer (OPL), the inner plexiform layer (IPL), and a retina homogenate (Hg).*

*$Na^+$ and temperature sensitive uptake of choline (Ch), aspartate (Asp), gamma-aminobutyric acid (GABA), and dopamine (DA) was demonstrated in OPL and IPL synaptosomes of rabbit retina. The uptake rate of all four compounds was lower in OPL synaptosomes compared to IPL synaptosomes : $^3$H-Ch 66%, $^3$H-GABA 48%, $^3$H-Asp 25% and H-DA 66.7 %.*

## Introduction

Most known putative neurotransmitters found in the central nervous system can also be isolated from retina. These include dopamine (DA), aspartate (Asp), glutamate (Glu) and glycine (Gly), gamma-aminobutyric acid (GABA) and acetylcholine (Ach).[1-5]

There is considerable evidence that uptake systems for neurotransmitters by nerve terminals play an important role in the overall process of chemical neurotransmission. In many cases, uptake is the mean by which neuronal transmission is terminated, and thus the efficacy of the uptake system can determine the duration of the transneuronal stimulation. In addition, many uptake systems are thought to play an important role in recycling a previously released putative transmitter, thus making it available for subsequent release.[6-9]

Yamamura and Snyder[10] have previously shown that high affinity choline uptake system is largely associated with synaptosomal fractions of the brain. The high affinity choline uptake is dependent on temperature, $Na^+$ concentration and metabolic

activity. A large percentage (62 - 70%) of the choline taken up by the high affinity system is used in Ach Synthesis.

The uptake of other known putative neurotransmitters appears to behave similarly to the choline uptake system with the exception of ACh. The components of these other high affinity uptake systems are also sensitive to temperature, $Na^+$ concentration, and metabolic rates (GABA[11] ; NE and DA[6] ; l - Asp[12] ; l - Glu[13] ). These other putative neurotransmitter systems have, likewise, been shown to be associated with synaptosomal fractions of the brain (DA and NE[6] ; GABA, Asp, Glu[14] ). Similar uptake systems for these putative neurotransmitters have been found in the retina (Asp, Glu[15] ; DA[16] ; ch[17] ; GABA[18] ).

In order to study the synaptosomal cholinergic uptake system Ch was used rather than ACh, because high affinity Ch uptake is specifically associated with cholinergic terminals for ACh synthesis ; ACh uptake is not. Instead, ACh is taken up in brain tissue by the low affinity systems of most neurons and glials and is not associated with a releasable pool [19 - 21]. The high affinity uptake rate for Ch has been shown, moreover, to be directly related to cholinergic neuronal activities *in vivo*[22,23]; an observation which indicates that Ch uptake is a dynamic and highly modifiable function. Redburn's isolation technique[24] makes it possible to separate large retinal synaptosomal fraction ($P_1$, mainly photoreceptor terminals from the outer plexiform layer, OPL of the retina) from small synaptosomes, ($P_2$, mainly nerve terminals from the inner plexiform layer, IPL, of the retina). This technique has recently been re-evaluated by Redburn and Thomas.[25] It was found that the $P_1$ fraction was substantialy enriched in photoreceptor synaptosomes from outer plexiform layer (OPL), as compared to other fractions. The photoreceptor synaptosomes are large (3 microns) and possess many synaptic vesicles and synaptic ribbons, usually surrounded by a halo of synaptic vesicles. In appropriate planes of section triad invaginations were seen which contained processes, presumably, from horizontal and bipolar cell neurites. The contaminations found in the $P_1$ fractions included some nuclei, inner and outer rod segments, and Muller cell fragments, which were often attached to the periphery of the photoreceptor synaptosomes. However, only about 11 % of the synaptosomes seen in this fraction were of the conventional type similar to those from the brain tissue.[25]

$P_2$ fractions taken from the retina were similar to the $P_2$ fractions obtained from whole brain[26]. In addition to the many conventional synaptosomes (1 micron or less) contained in this fraction, the $P_2$ had free mitochondria and empty membrane sacs. After comparing the samples with the morphology of the intact retina, Redburn and Thomas[25] concluded that the small synaptosomes were components of the inner plexiform layer (IPL). Electron microscopic analysis of the $P_2$ retinal fraction also showed a relatively large number of serial or reciprocal synaptic profiles. The availability of a technique which is capable of separating the $P_1$ (primarily from OPL) and $P_2$ (primarily from IPL)

synaptosomal fractions of the retina make it possible to study the basic characteristics of the uptake and release of putative neurotransmitter systems specifically associated with each plexiform layer.

Properties of neurotransmitter uptake systems of the retinal homogenate, $P_1$ and $P_2$ retinal synaptosomal fractions are reported here. In addition, calcium dependent-potassium stimulated release of one neurotransmitter is included. These investigations may give addition evidence of chemical neurotransmission process in retinal nerve terminals, especially, those in the outer and inner plexiform layers.

**Methods and Materials**

Adult, male New Zealand white rabbits (supplied by local commercial breeders in Houston, Texas, U.S.A.) weighing approximately 2 kilograms were used in all experiments. The animals were maintained under a 12 hour light/dark cycle in a temperature controlled room. They had free access to food and water, were allowed to adapt to the housing conditions for 3 days before they were sacrificed at 9-10 a.m. in the light adapted phase of the light cycle.

The details of retina subcellular fractionation procedures have been previously described by Redburn.[24] The $P_1$ and $P_2$ pellets which have been shown to contain mainly outer and inner plexiform synaptosomes[24] were resuspended in a small volume of sucrose (0.32M), and the samples were removed for protein determination using Lowry's technique.[27] Aliquots of homogenate (Hg), $P_1$ and $P_2$ were resuspended in oxygenated modified Ringer's solution (composed of 10 mM glucose, 20 mM N-2-hydroxyethylpiperazine N'-2-ethane-sulfonic acid (HEPES) ; 150 mM NaCl, 6.2 mM KCl, 1.2 mM $Na_2HPO_4 7H_2O$, with a pH adjustment to 7.4 using 10N NaOH) in a volume designed to insure a protein concentration of 0.1 - 0.2 mg/ml.

*A. Assay for Synaptosomal Uptake of Radioactive Labeled Putative Neurotransmitters and Related Compounds.*

The procedure used in this investigation of the uptake of putative neurotransmitters and related compounds in retinal and brain tissue follows that of Simon and Kuhar[22].

Oxygenated modified Ringer's solution, 0.9 mls, was added to 0.1 mls suspensions of retina or brain synaptosomal fractions. Samples of 150 µl were then removed and incubated at 37°C and 4°C for four minutes with various concentrations of either $^3$H-GABA (γ - aminobutyric acid, 35.1 curies/mmoles, New England Nuclear, NEN), $^3$H - DA (Dihydroxyphenylethylamine, dopamine, 16.6 Ci/mmole, NEN), $^3$H - Ch (13 Ci/mmole, Amersham Corporation, England), $^{14}$C - Asp (220 mCi/mmole, Amersham, England), or $^3$H - Glu (51.0 Ci/mmole, NEN). Uptake was terminated by rapid filtration through GR/A (Whatman) glass fiber filters mounted on a filter box (Fig. 1a). The trapped

synaptosomes were then rinsed with 2 x 4 ml of buffer at room temperature or at 4°C. The filters were placed in a plastic counting vial with 1 ml of 1% sodium dodecyl sulfate (SDS) in 20 mM of ethylenediamine tetra-acetic acid (EDTA) for 3-5 hours. Samples were counted by a Mark III scintillation counter, efficiency for $C^{14}$ was 65% and for $^{3}H$ was 40%, using 10 ml triton X-100-toluene base counting solution[28] : 250 mls triton X-100, 500 mls toluene, 2.25 gm 2,5-diphenyloxazole (PPO), 0.0225 gm 1,4-bis 2-(5-phenyloxazolyl) benzene ; phenyloxazolylphenyl-oxazolylphenyl (POPOP).

In experiments involving Ach or Ch 10 µM of neostigmine methyl sulfate (Sigma) was added to all buffers unless otherwise stated. In studies of $^{3}H$ - DA uptake, ascorbic acie (100 µM) and pargyline (10 µM) were uncluded in all buffers. Temperature dependent, high affinity uptake (uptake at 37° minus uptake at 4°C) of neurotransmitter related compounds was calculated as pmole/mg protein/4 min. of incubation. (To convert disintegration per minute (dpm) into pmole : pmole = 0.45 x dpm/specific activity in mCi/mmole for both $^{14}C$ and $^{3}H$ labeled compounds, 1 mCi = $2.22 \times 10^{9}$ dpm).

In some cases, the uptake was calculated as the ratio of the concentration in tissue to the concentration in the medium (T/M). This later calculation was performed by converting ligand uptake from pmole/mg protein to µmole/kg wet tissue which results in an approximation of µM, which can then be divided by the ligand concentration of the medium (µM). The resulting ratio indicates whether or not there is a net uptake of the ligand by the tissue.

*B. Assay for Release of Putative Neurotransmitters*

With only minor modifications, the procedures used for the putative neurotransmitter release study were those previously described by Levy *et al.*[29] Synaptosomal suspensions (about 0.5 mg protein/ml) were incubated in the presence of 0.5 µM labeled neurotransmitter or labeled Ch for 10 min. at 37°c. Synaptosomes were collected on GF/A glass-fiber filters (Whatman), mounted on a modified Swinnex filtering unit (Millipore Co.) (Fig. 1b), and rinsed with 4 ml of standard buffer at room temperature. Buffers (1 ml) containing different $K^{+}$ or $Ca^{++}$ concentrations were then dispensed onto the filter for a period of 45 seconds. A vacuum was applied for ten seconds, and the buffer was pulled through the filter into collections vials. After the vacuum was released (5 sec), fresh medium was added and the cycle was repeated. The sequence of buffers used in the assay was : 1) standard buffer (low $K^{+}$), 2) standard buffer plus 56.2 mM $K^{+}$ (High $K^{+}$), and 3) 56.2 mM $K^{+}$ + 3 mM $Ca^{++}$ (High $K^{+}$ plus $Ca^{++}$). Superfusates of 1 ml were collected at one minute intervals and the radioactive content was counted using the same scintillation fluid and counter previously described. A representative "release profile" of $^{3}H$-GABA from retina $P_1$ is shown in Fig. 2. Portion A of the figure represents the disintegration per minute (dpm) released when $Ca^{++}$ was added to the superfused medium. The amount of $Ca^{++}$ dependent potassium stimulated

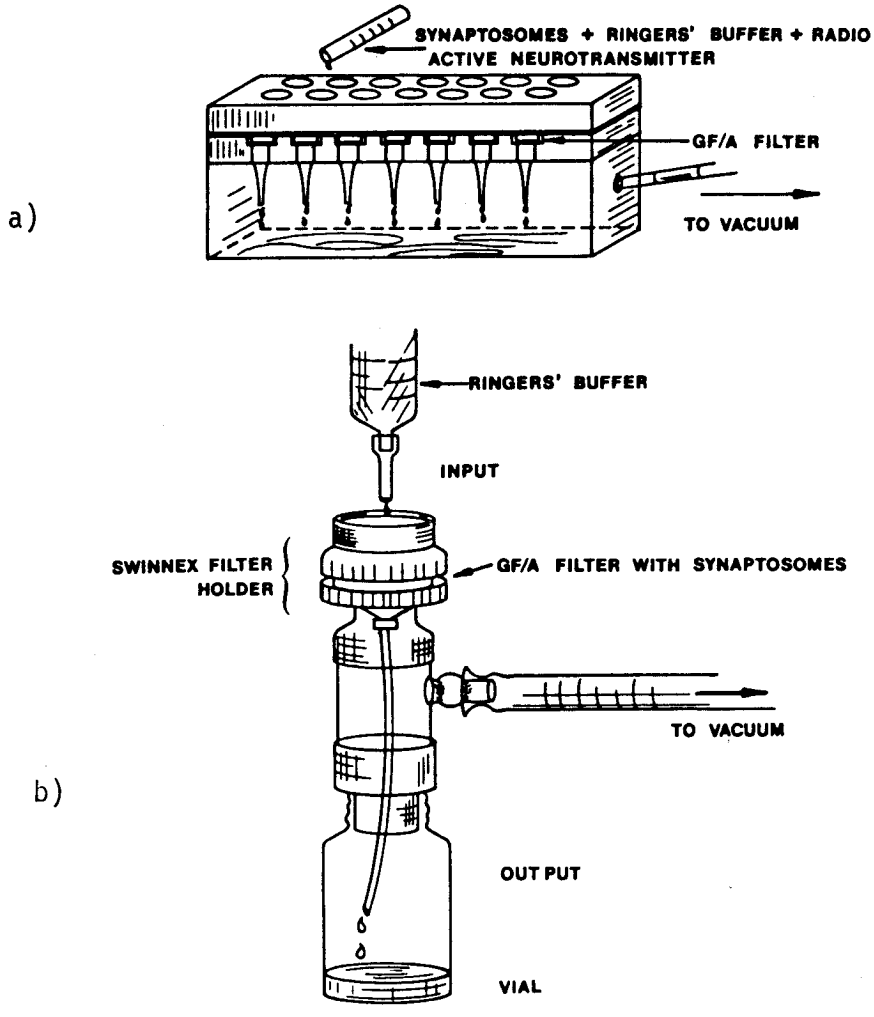


**Figure 1 (a).** Filter box used in uptake study. GF/A glass fiber filters were mounted in the slots clamped between the upper and lower lids where SWINNEX filter holders were fitted. It was then connected to a vacuum pump, which dried the filter and trapped the synaptosomes.

**(b).** Apparatus used for the neurotransmitter release study. A GF/A glass fiber filter (WHATMAN) was mounted in the modified SWINNEX filter holder. Synaptosomes containing radioactive neurotransmitters were trapped on the filter. Oxygenated buffers having different $K^+$ and $Ca^{++}$ concentrations were then added. Perfusates were collected in plastic vials by turning the vacuum pump on, and the system was temporarily depressurized. During the assay procedure, various media were layered on top of the filter for 45 seconds each ; a vacuum was then applied and the neurotransmitters was pulled through the filter into collection vials (10 sec.). After releasing the vacuum (5 sec.), fresh medium was added and the cycle was repeated.

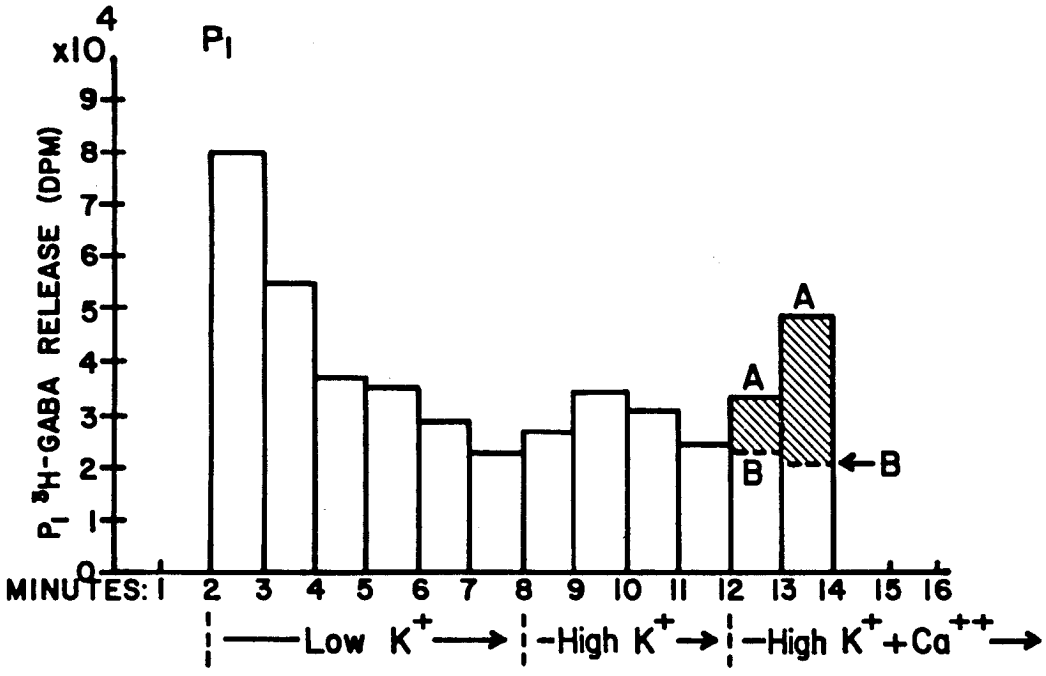


**Figure 2.** A representative "release profile" of $^{3}$H-GABA from $P_1$ synaptosomal fraction of retina. A synaptosome suspended in oxygenated normal buffer was incubated with $^{3}$H-GABA (0.5 μM) for 10 min, filtered on the GF/A filter mounted in the filtering unit (Fig. 1. b.), and washed with 4 ml of normal buffer Low ($K^+$). The perfusate was discarded, and the fractions were perfused first with normal Ringers' buffer (Low $K^+$), then with Low $K^+$ buffer + 50 mM KCl, (High $K^+$), and finally with High $K^+$ buffer + $CaCl_2$ 3 mM (High $K^+ + Ca^{++}$). One minute fractions were collected and counted, and the radioactivity was plotted as a function of the fraction number. On the right side of the graph, $Ca^{++}$ dependent release (shaded area) was calculated by substracting the baseline predicted for High $K^+$ release (B) from the actual radioactivity in High $K^+ + Ca^{++}$ (A). The percentage of reduction in spontaneous release in the High ($K^+$) fraction was used to determine the predicted baseline.

release was determined by subtracting B from A, and is shown as the shaded area in Fig. 2. In those cases where the B portion of the curve was not actually measured, values for B were extrapolated (see "Figure Legend" of Fig. 2 for calculation procedure).

The data obtained in the experiments was averaged and the mean ± standard error of mean (X ± SEM) are presented. All comparisons of data were done using student's paired t-test.

**Results**

*A. Kinetic Analysis of $^3$H-Ch and $^3$H-GABA Uptake by Retinal Fractions and the Initial Uptake Rates for $^3$H-DA, $^3$h-GABA, $^3$H-Ch and $^3$H-Asp.*

Most putative neurotransmitter systems in brain tissue have been shown to have high affinity uptake systems. These systems depend upon high concentrations of external $Na^+$ are temperature sensitive and show saturation kinetics. The uptake systems in the retina have also been shown to have characteristics similar to those of many brain areas. Uptake systems for Glu, Asp and DA studied by Thomas and Redburn[18] and Thomas et al[16] were also shown to be temperature and $Na^+$ dependent. Uptake was found to be linear with respect to time (0 - 10 min) and concentration (0.04 - 1.0 mg protein/ml).

Using the same experimental protocol and conditions, the characteristics of the high affinity uptake system in the retina for Ch and GABA were also investigated. Figures 3 (a) and 3 (b) demonstrate saturability of $^3$H-Ch and $^3$H-GABA uptake systems in retinal $P_1$ and $P_2$ fractions. The kinetic parameters obtained from the Lineweaver-Burk plot are summarized in Table 1. It can be seen that there is no significant difference between the $P_1$ and $P_2$ retinal synaptosomal fractions in the $K_T$ values of $^3$H Ch and $^3$H - GABA, whereas the Vmax of $^3$H - Ch and $^3$H - GABA of $P_2$ are significantly higher than those of $P_1$. Unless otherwise stated, 0.5 µM $^3$H-Ch and $^3$H-GABA was used in subsequent experiments, which is well below the $K_T$ values for uptake in both $P_1$ and $P_2$ fractions. Retinal synaptosomal µptake rates for Ch were also compared to those in synaptosomes from several other areas of the central nervous system (Unpublished data). Figure 4 shows the initial µptake rates of $^3$H - Ch by synaptosomal fractions of retina and several brain nuclei incubated with 0.5 µM $^3$H - Ch. The $^3$H-Ch uptake rate in $P_1$ retinal fraction is approximately 66 % of that of the $P_2$ rate. The $^3$H - Ch uptake rates for symaptosomal fractions of the lateral geniculate (LGN), superior colliculus (SC), visual area (VA), were found to be lower than those of caudate nucleus (CN) and hippocampus (HPC) (unpublished data).

Simultaneous studies of the uptake rates of $^3$H - GABA, $^3$H - DA, $^{14}$C Asp and $^3$H - Ch by homogenate, $P_1$ and $P_2$ retinal fractions are shown in Fig. 5. The uptake rates at 0.5 µM for these ligands are also higher in the $P_2$ fraction than in the $P_1$ fractions. Although the $P_1$ uptake rate for $^{14}$C - Asp, $^3$H-Ch, $^3$H-GABA and $^3$H-DA is lower than that of $P_2$, there is clear evidence for active uptake of these compounds.

The tissue to medium ratios of ligand concentration (T/M ratios) calculated from the above data are shown in Table 2. It can be seen that in nearly all cases the ligand concentration in the tissue fraction is higher than that in the incubating media. $^{3}$H-Ch uptake by retinal homogenate and lateral geniculate nucleus synaptosomal fractions and the retinal homogenate uptake of $^{3}$H-DA, however, have T/M ratios of about one. When the T/M ratios in Table 2 are correlated with the data in Figs. 6 and 7, it can be seen that ligands with high T/M uptake ratios are, in general, more sensitive to temperature in the incubation medium than are those with low T/M ratios.

*B. Temperature and $Na^{+}$ Dependency of $^{3}$H-Ch, $^{14}$C-Asp, $^{3}$H-GABA and $^{3}$H-DA Uptake by Homogenate, $P_1$ and $P_2$ Synaptosomal Fractions of the Retina.*

As is true for most neuronal tissue, the uptake of putative neurotransmitters by the the retinal synaptosomal fractions $P_1$, $P_2$ and retinal homogenate is both $Na^{+}$ and temperature sensitive (Figs. 6, 7 and 8). The degree of sensitivity to these factors varies, however, from one structure to another. It can be seen in Fig. 6 that the uptake of $^{3}$H - Ch synaptosomal fractions of the caudate nucleus, the hippocampus and the retinal $P_2$ fraction was significantly reduced when the incubation temperature was lowered. However, uptake of $^{3}$H - Ch by retinal homogenate, $P_1$ retinal fraction and the synaptosomal fractions of the lateral geniculate nuclei, superior colliculi and visual cortex was not as much reduced. Figure 7 provides a comparison with similar experiments for $^{14}$C - Asp, $^{3}$H - GABA and $^{3}$H - DA. In general, the uptake system for these ligands in the $P_2$ retinal fraction is more temperature dependent than that in the $P_1$ and Hg retinal fractions. Figure 8 illustrates the $Na^{+}$ dependency of high affinity uptake of $^{3}$H - GABA, $^{3}$H - Asp and $^{3}$H - Glu. The figure demonstrates that when 150 mM NaCl is omitted from the media and replaced with 300 mM sucrose the uptake of putative neurotransmitters is significantly reduced in both the $P_1$ and $P_2$ fractions.

*C. $Ca^{++}$ Dependent Release of $H^{3}$ - GABA from the $P_1$ Fraction.*

The release of all known putative neurotransmitters from synaptosomal fractions is calcium dependent. Experiments on retinal synaptosomal fractions (Fig. 2) demonstrate that the release of $^{3}$H-GABA from $P_1$ is also calcium dependent.

## Discussion

Uptake systems in the retina, particularly those in the outer plexiform layer (OPL), may be functionally important in the visual process. Several indirect lines of evidence indicate that, in the dark, photoreceptor cells tonically release an excitatory neurotransmitter. Exposure to light produces a decrease in the release of excitatory neurotransmitters and this can trigger the visual processes of the retina[30 - 31] Since the visual response in the OPL is triggered by a decrease in the release of an excitatory transmitter from photoreceptors, the previously released neurotransmitter must be removed (taken up or destroyed before the postsynaptic cells can respond to light stimulation. The rate of

**TABLE 1.** $K_T$ AND Vmax OF HIGH AFFINITY $^3H$ - CHOLINE AND $^3H$ - GABA UPTAKE IN RABBIT RETINA.

The $K_T$ and Vmax were calculated from the same data used in Fig. 3, N values are shown in parentheses.

| | $K_T$ (μM) | Vmax (p mole/mg prot./4 min). |
|---|---|---|
| $^3H$ - Ch uptake | | |
| $P_1$ | 0.97 ± 0.65 | 13.03 ± 1.66 (6) |
| $P_2$ | 0.85 ± 0.42[a] | 46.28 ± 16.66 (6)[b] |
| $^3H$-GABA uptake | | |
| $P_1$ | 4.83 ± 1.42 | 1204.91 ± 345.28 (4) |
| $P_2$ | 5.86 ± 0.84[a] | 4399.16 ± 905.19 (4)[b] |

[a] *no significane*
[b] *significant difference (P < 0.01)*

**TABLE 2.** TISSUE TO MEDIUM (T/M) RATIO OF PUTATIVE NEUROTRANSMITTERS AND ONE OF THEIR PRECURSORS (X ± SEM) AT 0.5 μM.

The ligands were incubated for 4 minutes at 37°C (at 4°C).

| T/M ratio of uptake of | Hg | $P_1$ | $P_2$ |
|---|---|---|---|
| $^3H$-Ch | 0.82 ± 0.17 | 3.07 ± 0.96[a] | 4.21 ± 0.65[a] |
| $^{14}C$-Asp | 4.95 ± 1.67 | 9.13 ± 1.41 | 22.42 ± 2.02[a] |
| $^3H$-GABA | 20.74 ± 3.25 | 45.70 ± 10.30[a] | 79.16 ± 16.53[a] |
| $^3H$-DA | 1.01 ± 0.19 | 2.66 ± 0.88[b] | 2.23 ± 0.33[a] |

[a] *highly significant defference (P < 0.01)*
[b] *significant difference (P < 0.05)*

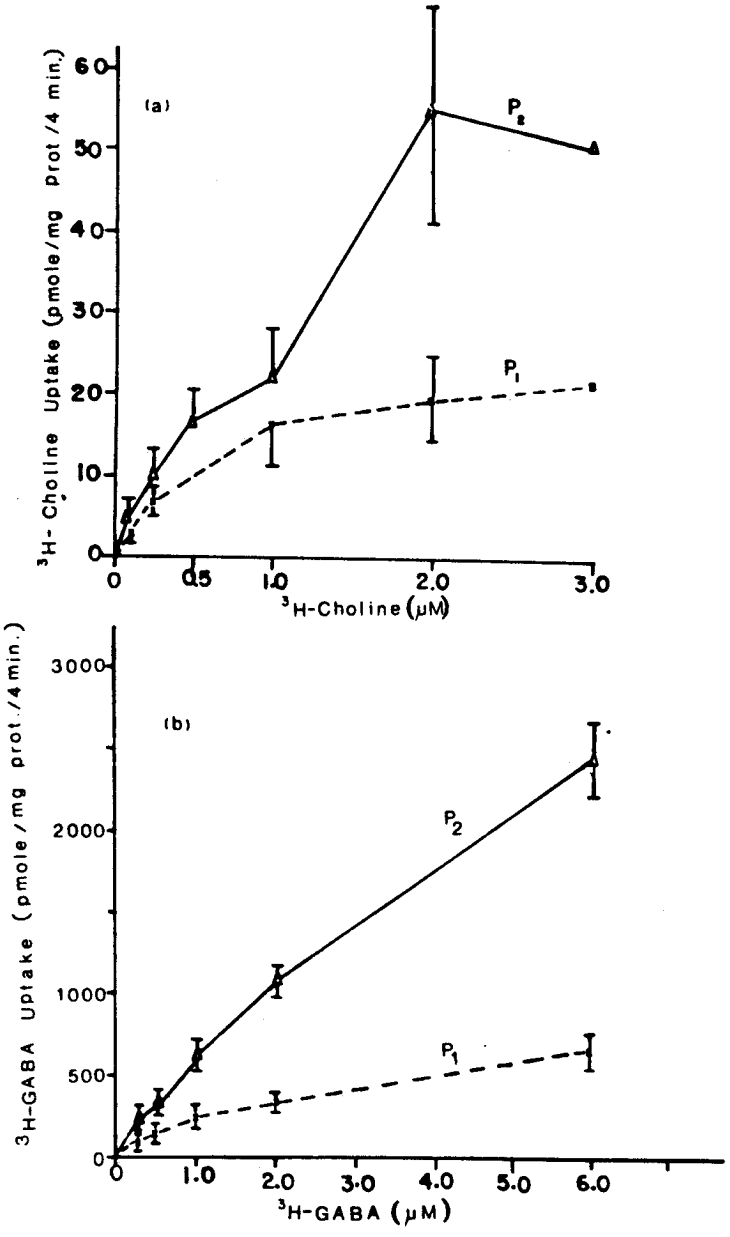


**Figure 3.** Uptake of $^3$H - Ch and $^3$H - GABA. Subcellular fractions, ($P_1$ and $P_2$), were suspended in oxygenated modified Ringers' buffer which gave a final protein concentration of approximately 0.2 mg/ml, and were incubated with several different concentrations of $^3$H - Ch or $^3$H - GABA for 4 minutes at 37°C. Samples were rapidly filtered, rinsed, solubilized and counted. Uptake at 4°C was substracted from uptake at 37°C. The data presented are the means obtained ± S.E.M. from separate preparations (for $^3$H-Ch uptake N = 6, each N = a pool of 6 eyes; for $^3$H-GABA uptake, N = 4, each N = a pool of 2 eyes).

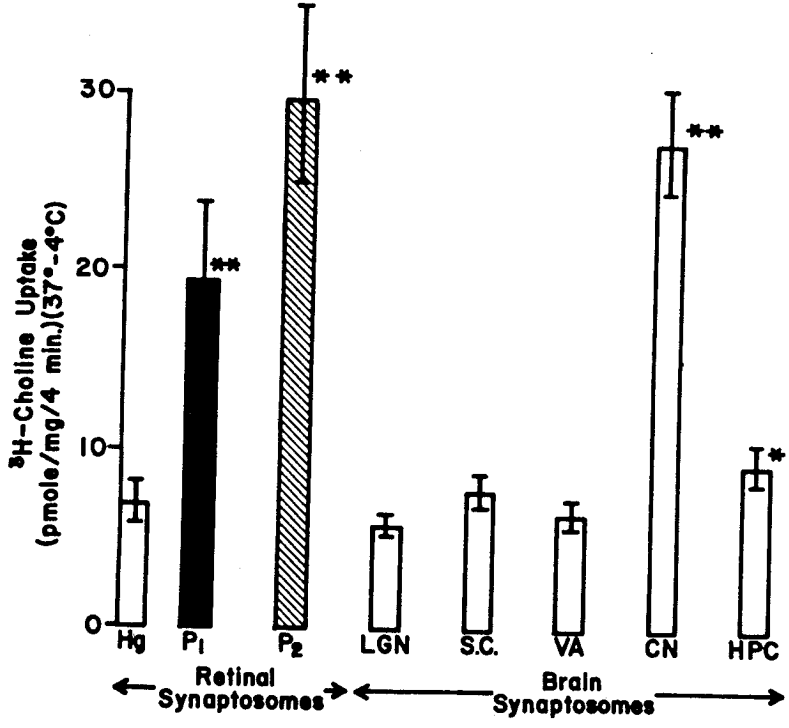


**Figure 4.** High affinity uptake of $^3$H - Ch at 0.5 μM in retinal homogenate, $P_1$ and $P_2$ and in synaptosomal fractions of several areas of the brain : lateral geniculate nucleus (LGN), superior colliculi (S.C.), visual areas I and II (VA), caudate nucleus (CN), and hippocampus (HPC). Experimental conditions are the same as those in Fig. 3. **Designates a highly significant difference ($P<0.01$) of $^3$H - Ch uptake from retina homogenate. (For eyes : N = 6 each N = a pool of 4 eyes ; for brain tissues : N = 6, each N = tissue from 1 brain).

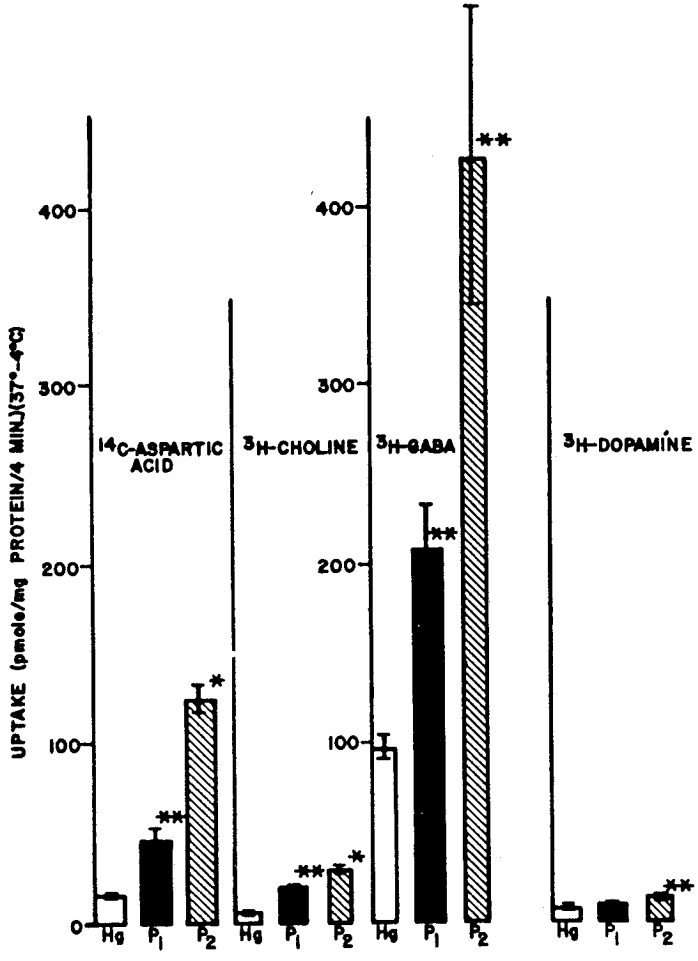


**Figure 5.** Comparisons of high affinity uptake rates for $^{3}H$ - Asp, $^{3}H$ - Ch, $^{3}H$ - GABA and $^{3}H$ - DA, 0.5 uM in 4 minute incubations by the $P_1$ and $P_2$ fractions of the retina. Experimental conditions are similar to those in Fig. 3. * and ** designates significant, $P < 0.05$ and highly significant differences, $P < 0.01$, between the uptake rate of $P_1$ and $P_2$ and the retina homogenate. (N = 6, each N = a pool of 4 eyes.).

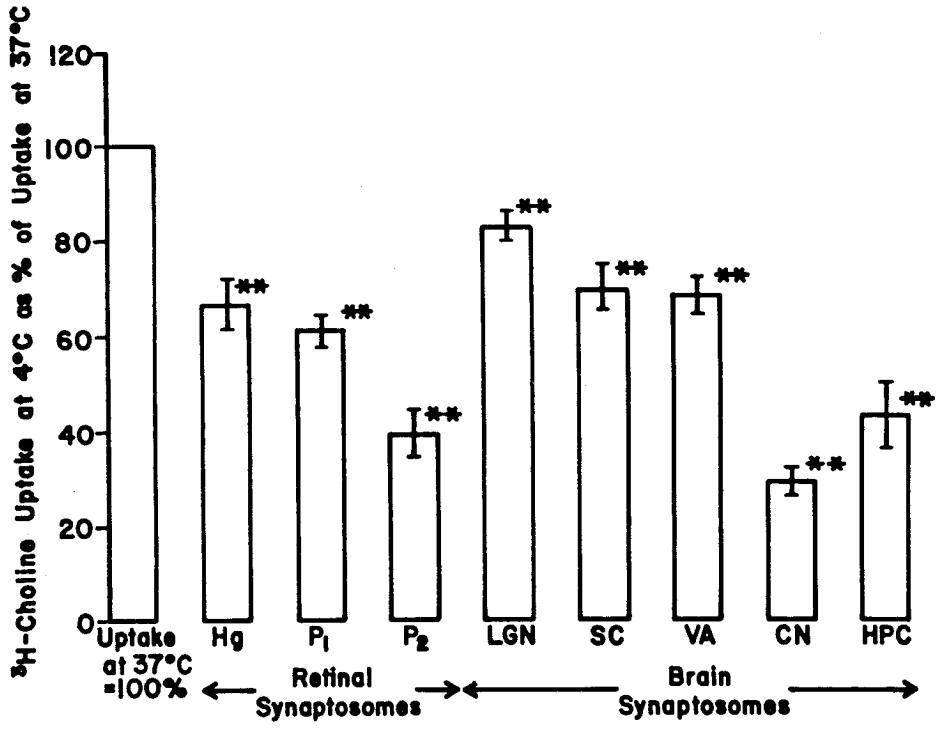


**Figure 6.** Effect obtained by lowering the temperature from 37° C to 4° C on the $^3$H - cho high affinity uptake rate of retina homogenate, $P_1$ and $P_2$ and on synaptosomes from several areas of the brain. $^3$H - Ch concentration used was 0.5 uM with a four minute incubation time. $^3$H - Ch, uptake at 4° C was calculated as a percentage of tne values obtained at 37° C

** designates highly significant differences (or $P<0.01$) between uptake at 4° C. (For eyes : N = 6, each N = a pool of 4 eyes ; for brain : 4 = 6, each N = a tissue from 1 brain).

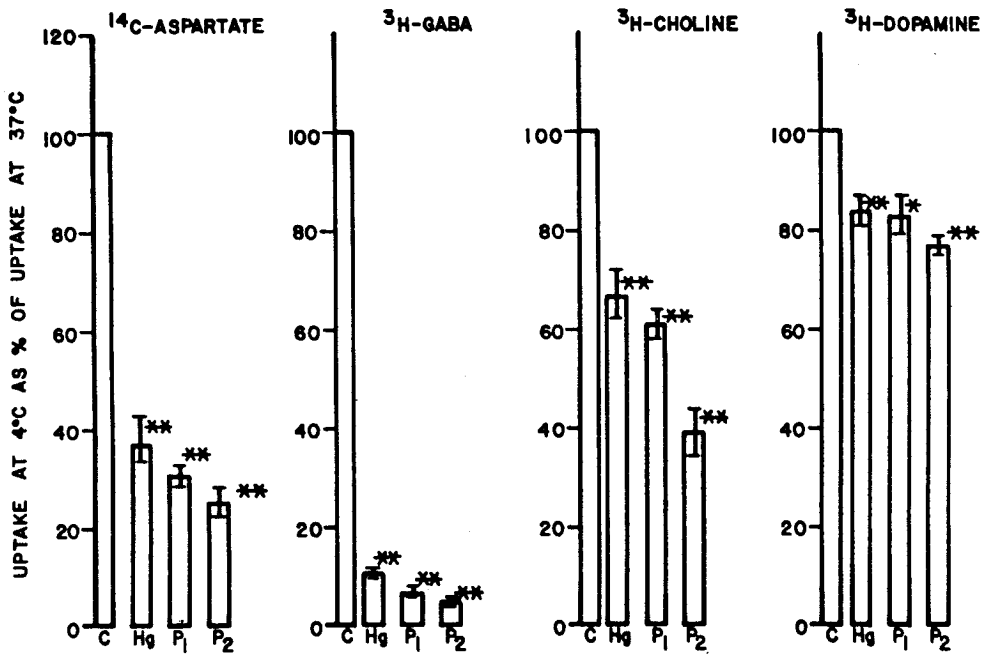


**Figure 7.** A comparison of the temperature dependency of high affinity uptake of $^{14}$C-Asp , $^{3}$H - GABA, $^{3}$H - Ch and $^{3}$H - DA by homogenate, $P_1$ and $P_2$ retinal fractions. Concentration of labeled compounds in the incubations was 0.5 UM with an incubation time of four minutes. Experimental conditions are himilar to those in Fig. 6.

** designates highly significant differences between uptake of the the retinal fraction at 4° C and 37° C.

(N = 6, each N = a pool of 4 eyes).

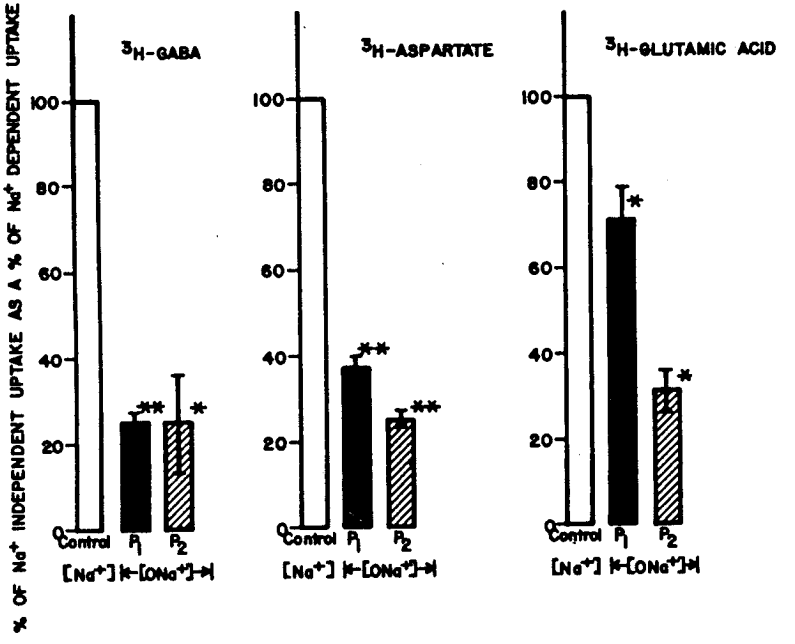


**Figure 8.** $Na^+$ dependency of the uptake of $^3H$ - GABA, $^3H$ - Asp and $^3H$ - Glu by $P_1$ and $P_2$ retinal fractions. Ligand concentrations were maintained at 0.5 uM during a four minute incubation period. Uptake of labeled putative neurotransmitter at O $Na^+$ was calculated as percentage of the uptake at normal $Na^+$ concentration (150 mM). Sucrose (130 mM) was added to replace $Na^+$ in the $Na^+$ free buffer (O $Na^+$).
and designate $P<0.05$ and $P<0.01$ respectively between uptake at normal ($Na^+$) and 0 ($Na^+$).
(N = 3, each N = a pool of 2 eyes).

neurotransmitter reuptake in the retina may, therefore, determine the time required for bipolar and horizontal cells to respond to light induced changes in the photoreceptor cell. Studies on the characteristics of neurotransmitter uptake systems in the retina provide important information concerning neuronal processing in the visual system.

Some of the well-known characteristics of high affinity uptake for putative neurotransmitters in central nervous system tissue are found here in retinal synaptosomal fractions. These characteristics include saturability, $Na^+$ dependency and temperature dependence. Figures 3 (a) and 3 (b) show the saturability of $^3H$ - Ch and $^3H$ - GABA high affinity uptake in retinal $P_1$ and $P_2$ fractions. The maximal rate of uptake (Vmax) for $^3H$ - Ch and $^3H$ - GABA are highest in $P_2$. $K_T$ (Michaelis transport constant) values for high affinity choline uptake by rabbit retina $P_1$ and $P_2$ (0.97 ± 0.65, and 0.85 ± 0.42 μM, respectively) are not significantly different from those obtained for intact rat retina (2.0 μM). Values reported for low affinity transport systems are much higher (100 μM)[17]. Low affinity uptake systems were not investigated in this study. $K_T$ of $^3H$-GABA uptake by $P_1$ and $P_2$ are not significantly different (4.83 ± 1.42 and 5.86 ± 0.84 μM, respectively).

Kuhar *et al*[20], as well as Carroll and Buterbaugh[34], have found that the Vmax for high affinity choline uptake in regional brain synaptosomal fractions reflects density of cholinergic nerve terminals within a given brain region. If these findings hold true for the retina, the $P_2$ retinal synaptosomal fractions, and by extension, the IPL should have a higher density of cholinergic nerve endings than the $P_1$ fraction and the OPL. This hypothesis is supported by the data in Figure 3 (a) which shows that the Vmax for $^3H$ - Ch uptake in $P_1$ and $P_2$ correlate with the distribution of other cholinergic markers in the retina (Rabbit retina[35 - 38]).

Table 2. indicates the tissue/medium ratios (T/M) for accumulated ligand concentrations in tissue vs. ligand concentrations in medium. These values were taken after incubation with 0.5 μM ligand for 4 min at 37°C. The data indicates that there is net active uptake of putative neurotransmitters or precursor (Ch) into the synaptosomal fractions of retina, with the exception of uptake of Ch and Da into the retinal homogenates, and the Ch into lateral geniculate nucleus synaptosomal fraction. This may suggest that nerve ending density in these latter populations is too low to demonstrate net uptake in 4 min of incubation.

Table 2. also shows that retinal or brain synaptosomal fractions which have high T/M uptake ratios for particular ligands also have highly temperature dependent uptake systems for those ligands. (Fig. 6 and 7). The data suggest, therefore, that the uptake systems studied in this investigation are active transport systems.

The $Na^+$ dependency of the retinal uptake systems has been previously reported for $^3H$ - GABA, $^3H$ - Asp (Fig. 8), $^3H$ - Ch (rat and rabbit[17] ; chicken[39], $^3H$ -DA (rabbit[16]) and $^3H$ - GABA (rat[40]). The $K_T$ value obtained for $^3H$ - GABA uptake by $P_1$ synaptosomal fractions of rabbit retina is 4.83 ± 1.42 μM and that by $P_2$ is 5.86 ± 0.84 μM. These are lower than the $K_T$, 40 μM, obtained from whole rat retina.[18] $Ca^{++}$ dependent release of $^3H$-GABA from the $P_1$ retinal fraction has also been demonstrated (Fig. 2). This indicates that the $^3H$-GABA taken up by the $P_1$ fraction enters a pool which is released by $Ca^{++}$ stimulated secretion coupling mechanisms.

In conclusion, the uptake of putative neurotransmitter related compounds in rabbit retinal homogenates and in the $P_1$ and $P_2$ retinal fractions show characteristics which are similar to those shown by synaptosomal fractions from the brain. These characteristics include saturable kinetics for $^3H$ - Ch and $^3H$ - GABA, as well as $Na^+$ and temperature dependency. The tissue to medium (T/M) ratios shows a net active uptake of $^3H$ - Ch, $^3G$ - GABA, $^3H$ - DA and $^{14}C$ Asp in every retinal fraction studied. The release of $^3H$-GABA is stimulated by depolarizing levels of potassium in the presence of calcium. The existence of cholinergic and GABAergic $P_1$ fraction is indicated. Relative to $P_2$, $P_1$ shows relatively minor, but significant, uptake systems for $^3H$ - Ch and $^3H$ - GABA.

**Acknowledgements**

This project was supported by the NEI grant EYO-1655 - 02 (DAR) and the Rockefeller Foundation (TC). Thanks are due to Miss. Suthada Homjun for her secretarial help.

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## บทคัดย่อ

ส่วนเชื่อมต่อของปลายประสาท (synapse) ในเรตินาม่ีระบบดูดสารนิวโรทรานสมิตเตอร์กลับ ซึ่งเป็นส่วนสำคัญอันหนึ่งในกระบวนการส่งสัญญาณประสาท. ได้ทำการทดลองเปรียบเทียบระบบดูดซึมนิวโรทรานสมิตเตอร์กลับโดยซินแนบโตโซมหรือชิ้นส่วนปลายประสาทที่เตรียมได้จากปลายประสาทชั้นนอก (Outer plexiform layer, OPL) ปลายประสาทชั้นใน (Inner plexiform layer, IPL) และส่วนบดละเอียด (Homogenate) ของเรตินา. ได้พบว่าการดูดซึมโคลีน, แอสปาเตท, แกมม่า - อะมิโนบูทีริคแอซิด, และ ดอปปามีนในปลายประสาทจากชั้นนอก (OPL) จากเรตินาของกระต่ายต้องการใช้โซเดียมไอออนส์และไวต่อการเปลี่ยนแปลงอุณหภูมิ อัตราการดูดซึมของสารสี่ตัวดังกล่าวโดยปลายประสาทชั้นนอก (OPL) ช้ากว่าปลายประสาทชั้นใน (IPL). คือ $^{3}$H- โคลีน 66 %, $^{3}$H- แกมม่าอะมิโนบูทีริคแอซิค 48 %, $^{3}$H- แอสปาเตท 25% และ $^{3}$H- ดอปปามีน 66.7 % ของปลายประสาทชั้นนอกเมื่อเทียบกับปลายประสาทชั้นในของเรตินา.