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The uptake of ^{3}H-γ-aminobutyric acid by the retina

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Summary

1. The accumulation of ^{3}H-γ-aminobutyric acid (GABA) by the isolated rat retina has been measured.

2. When retinae were incubated at 37° C in a medium containing ^{3}H-GABA, tissue:medium ratios of about 25:1 were attained after a 30 min incubation.

3. After incubations of 40 min at 37° C, almost all (98%) the radioactivity in the tissue was present as unchanged ^{3}H-GABA.

4. The process responsible for ^{3}H-GABA uptake showed many of the properties of an active uptake system: it was temperature-sensitive, required the presence of sodium ions in the external medium, was inhibited by anoxia, dinitrophenol and ouabain, and showed saturation kinetics.

5. The estimated Km value of GABA was $4{\cdot}0 \times 10^{-5}$M, and V_{max} was 0·167 (μmoles/min)/g retina.

6. The uptake of ^{3}H-GABA was not affected by the presence of large molar excesses of glycine, L-glutamate, L-aspartate, L-alanine, L-proline, or L-histidine, but was inhibited by DL-γ-amino-β-hydroxybutyrate, β-guanidinopropionate, and L-2,4-diaminobutyrate.

7. The retina was capable of achieving a large net uptake of GABA, indicating that the accumulation of ^{3}H-GABA by the tissue was not due only to an exchange process with the endogenous GABA pool.

8. The uptake of ^{3}H-GABA occurred only in tissue from the central nervous system. Thus, retina and cerebral cortex rapidly accumulated radioactivity, but slices of cornea, posterior wall of the eye, and liver achieved tissue: medium ratios of approximately one.

9. There was a rapid efflux of radioactivity from retinae placed in fresh medium and after 60 min, 90% of the radioactivity was lost from the tissue. The radioactivity released into the medium was present largely as ^{3}H-acidic and neutral metabolites. When the metabolism of GABA was inhibited by the presence of amino-oxyacetic acid in the medium, only about 10% of the radioactivity was lost from the tissue during a similar 60 min incubation, and the radioactivity released was present largely as unchanged ^{3}H-GABA.

10. It is suggested that the GABA uptake process may represent a possible mechanism for the inactivation of GABA if this amino acid is released at inhibitory synapses in the retina.

Introduction

In vertebrates, γ-aminobutyric acid (GABA) occurs in significant quantities only in the central nervous system, where it is probably an important synaptic inhibitory

transmitter substance (Krnjević & Schwartz, 1967 ; Obata, Ito, Ochi & Sato, 1967 ; Krnjević, 1970 ; Hebb, 1970). GABA is present in retina in amounts similar to those found in cerebral cortex (1–3 μmol/g) (Kojima, Mizuno & Miyazaki, 1958 ; Kuriyama, Sisken, Haber & Roberts, 1968 ; Graham, Baxter & Lolley, 1970). Furthermore, GABA has been shown to have an uneven distribution across the different layers of the frog and rabbit retina and it appears to occur in particularly high concentrations in amacrine cells and horizontal cells (Graham *et al.*, 1970 ; Graham, 1972). Since these cells may have an inhibitory function in the retina (Dowling, 1967), it is possible that GABA may be the inhibitory transmitter substance released from their presynaptic terminals. Consistent with this suggestion are neuropharmacological studies which have shown that GABA applied to the retina *in vitro* or *in vivo* inhibits both spontaneous and light-induced electrical activity (Kishida & Naka, 1967 ; Ames & Pollen, 1969 ; Straschill, 1968 ; Straschill & Perwein, 1969) ; also the parenteral administration of GABA depresses the b-wave of the electroretinogram of young chicks (Kramer, Sherman & Seifter, 1967 ; Scholes & Roberts, 1964).

The mechanism by which the inhibitory effects of applied GABA or neurally released GABA on neurones are terminated is not clear ; however, brain tissue possesses the ability to concentrate GABA from an external medium (Elliott & van Gelder, 1958 ; Iversen & Neal, 1968) and it has been suggested that GABA, and other amino acid transmitters, following their release from inhibitory nerve-endings, might be inactivated by a re-uptake process (Iversen & Neal, 1968 ; Neal & Pickles, 1969 ; Curtis, Duggan & Johnston, 1970 ; Neal, 1971). Recent autoradiographic studies have confirmed that the retina is capable of accumulating radioactive GABA (Ehinger, 1970 ; Ehinger & Falck, 1971 ; Lam & Steinman, 1971 ; Neal & Iversen, 1972) and the present experiments were undertaken to establish the properties of the GABA uptake system in the mammalian retina. Preliminary results of these studies have been reported previously (Goodchild & Neal, 1970) and have recently been confirmed by Starr & Voaden (1972).

Methods

Uptake of ^{3}H-γ-aminobutyric acid by rat retina

Male Wistar rats (180–240 g) were light adapted by placing their cage in daylight for at least 2 h and then killed by cervical dislocation. The eyes were enucleated rapidly and opened at the corneoscleral junction. Each retina (approximately 10 mg wet weight) was rapidly dissected under ice-cold incubation medium, weighed and placed in a 25 ml conical flask containing 9·5 ml of ice-cold incubation medium. The flasks were gassed with a mixture of oxygen (95%) and carbon dioxide (5%) and fitted with rubber seals. The retinae were given a preliminary incubation for 15 min in a shaking water bath ; then 0·5 ml of incubation medium containing ^{3}H-GABA was injected through the rubber seals, and the incubations were continued for various times. Unless otherwise stated, preliminary incubation and incubations were performed at 25° C, which was found to be the optimal temperature for ^{3}H-GABA accumulation. The final concentration of ^{3}H-GABA in the incubation medium was $5{\cdot}0 \times 10^{-8}$M in most experiments. The tissue was recovered by rapid filtration on a small Buchner funnel fitted with a Whatman No. 1 filter paper disc (2·0 cm diameter), and washed twice with 5 ml of ice-cold

incubation medium. The filter discs with the retinae, were transferred to counting vials and the ^{3}H-GABA was extracted by dissolving the tissue in 1·0 ml of Soluene Tm 100 (Packard). The radioactivity was estimated by liquid scintillation spectrometry after the addition of 10 ml of phosphor (1% butyl PBD, Ciba, in toluene).

Preliminary experiments established that there was no significant loss of ^{3}H-GABA from the retinae during the washing procedure and that the wash removed most of the radioactivity in the incubation medium remaining on the filter paper. The results were corrected for the presence of the small amount of radioactivity which remained on the filter paper after washing (0·4 nCi).

Net uptake of γ-aminobutyric acid by rat retina

In some experiments, two retinae (approximately 20 mg wet weight tissue) were given a preliminary incubation for 15 min in 10 ml of incubation medium, then ^{3}H-GABA was added to give a final concentration of 5×10^{-4}M and the incubation was continued for 30 minutes. The retinae were recovered with forceps and washed for 2 min by immersion in 10 ml of ice-cold incubation medium. The tissue was then homogenized in 1·0 ml of hydrochloric acid (0·1 M) and left at 0–4° C for 1 hour. The homogenate was transferred to a centrifuge tube and the homogenizer was washed with a further 0·5 ml of hydrochloric acid. The homogenate, together with the washing fluid, was centrifuged (1,000 g × 15 min) to remove tissue debris. Samples of the supernatant (50 μl) were taken for scintillation counting. The remaining fluid was heated in a boiling water bath for 3 min and recentrifuged to remove the protein. The amino acid composition of the final supernatant was determined with an automatic amino acid analyser (Biocal BC 100).

Efflux of ^{3}H-γ-aminobutyric acid from rat retina

Retinae were incubated individually with ^{3}H-GABA (5×10^{-8}M) for 30 min at 25° C and then recovered with forceps. The retinae were washed by immersion in 10 ml of fresh ice-cold medium for 2 min, and then each was placed in 5 ml of fresh medium at 25° C. The retinae were incubated in a shaking water bath for 60 minutes. The release of radioactivity was determined by removing 0·2 ml samples from the medium at various times. The incubation volume was kept constant by adding 0·2 ml of fresh medium immediately after sampling. On completion of the incubation, the tissue was recovered and dissolved in 1·0 ml of Soluene Tm 100. The radioactivity which had remained in the tissue was then estimated by liquid scintillation counting.

The influence of GABA metabolism on the efflux of radioactivity from the retina was investigated by repeating the efflux experiments with medium containing aminooxyacetic acid (0·1 mM). This substance inhibits 4-aminobutyrate-2-oxoglutarate aminotransferase (GABA-T) which is the only enzyme present in the central nervous system which is capable of effecting the metabolic degradation of GABA.

In some experiments the medium was acidified at the end of the 60 min incubation period and subjected to ion-exchange chromatography (Otsuka, Iversen, Hall & Kravitz, 1966). This method results in the separation of three fractions: (1) acid and neutral metabolites not retained on Amberlite CG120 resin in the H^+ form, (2) acidic amino acids retained on Dowex 1 resin in the acetate form, and (3) GABA

and glutamine which are not retained on Dowex-1-acetate resin. GABA was not separated from glutamine in these experiments.

Materials

The incubation medium was Krebs-bicarbonate Ringer of the following composition (g/l.): NaCl, 6·92 ; KCl, 0·354 ; $CaCl_2$, 0·28 ; $MgSO_4$, 0·144 ; KH_2PO_4, 0·162 ; $NaHCO_3$, 2·1 ; D-glucose, 2·0. GABA-2,3-[^{3}H], specific activity=2 Ci/mmol was obtained in a chromatographically pure form, from New England Nuclear Chemical GmbH, 6072, Dreieichenhain, West Germany. D-Mannitol-1-[^{14}C], specific activity 30 mCi/mmol, and inulin-[^{3}H], specific activity 300 mCi/mmol, were obtained from the Radiochemical Centre, Amersham, England. Amino-oxyacetic acid (H_2NOCH_2COOH) was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wisconsin.

Results

Uptake and metabolism of ^{3}H-γ-aminobutyric acid

When individual retinae were incubated at 37° C in a medium containing ^{3}H-GABA (5×10^{-8}M) there was a rapid accumulation of radioactivity in the tissue resulting in a maximum tissue/medium ratio (^{3}H-GABA dpm/g tissue: ^{3}H-GABA dpm/ml of medium) of 25:1 after a 40 min incubation (Fig. 1). Because of the high tissue/medium ratios attained, it was necessary to use a large volume of incubation medium (10 ml) in relation to the amount of tissue (10 mg) and under these conditions the concentration of ^{3}H-GABA in the incubation medium fell by less than 6% during a 40 min incubation. The extracellular space in the retina was estimated by incubating the tissue with ^{3}H-inulin or ^{14}C-mannitol (Fig. 1). The spaces obtained with these substances were $43 \pm 7\%$ and $52 \pm 4\%$ respectively (mean $\pm$ S.E. of mean of 4 experiments). The results were not corrected for the small amount of radioactivity accumulated in the extracellular space.

In six experiments in which retinae were incubated for 40 min, tissue extracts were concentrated and subjected to descending paper chromatography, with ammonia : phenol : water as the solvent system (phenol saturated with water 99·5%, concentrated ammonia solution 0·5% v/v, 8-hydroxyquinoline, 0·1% w/v). Only a single radioactive spot, corresponding in Rf to authentic GABA was detected. The tissue protein remaining after the extraction procedure was dissolved in Soluene Tm 100 and the radioactivity was measured; less than 1% of the ^{3}H-GABA was incorporated into the protein.

In subsequent experiments, therefore, the uptake of ^{3}H-GABA was measured by determining the accumulation of total radioactivity in the tissue.

Time-course of ^{3}H-γ-aminobutyric acid uptake

The time course of ^{3}H-GABA accumulation in isolated retinae incubated at 37° C with ^{3}H-GABA (5×10^{-8}M) is shown in Figure 1. There was an initial phase of very rapid uptake (0–10 min) which was almost linear for the first 5 minutes; this was followed by a slower phase of uptake (10–30 minutes). The maximum accumulation of ^{3}H-GABA occurred after 40–50 min and a decline in the accumulation of ^{3}H-GABA occurred when the retinae were incubated for a longer time (60 minutes).

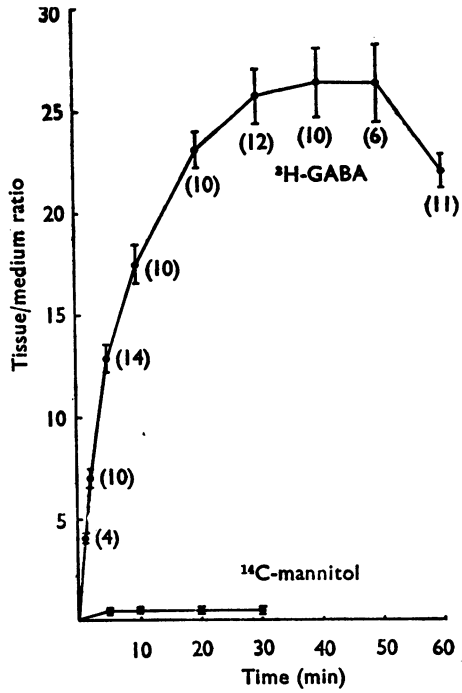

FIG. 1. Time course of ^{3}H-γ-aminobutyric acid (GABA) uptake in retinae incubated at 37° C with ^{3}H-GABA (5×10^{-8}M). Bottom line shows uptake of ^{14}C-mannitol in retinae incubated at 37° C with ^{14}C-mannitol (0·1 μCi/ml). Figures in parentheses indicate number of experiments at each time; vertical bars indicate S.E.M.

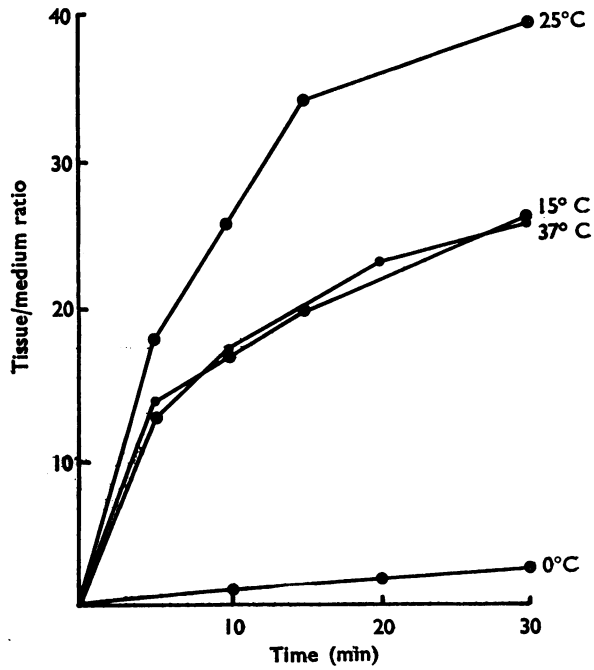

FIG. 2. Effect of temperature on ^{3}H-γ-aminobutyric acid (GABA) uptake by retinae incubated with ^{3}H-GABA (5×10^{-8}M). Each point is the mean of at least six experiments.

Effect of temperature on ^{3}H-γ-aminobutyric acid uptake

The optimal temperature for the accumulation of ^{3}H-GABA by the tissue was 25° C (Fig. 2) and this temperature was used for subsequent experiments. When incubations were performed at 0° C the rate of ^{3}H-GABA uptake was markedly reduced when compared with that at higher temperature. However, some uptake of ^{3}H-GABA still occurred at this temperature, giving a tissue medium ratio of 2·5:1 after a 30 min incubation. Incubations at 15° C and 37° C give similar results. The values obtained at 25° C and 15° C indicate a Q_{10} of approximately 1·8 for the uptake process in this temperature range.

Effect of γ-aminobutyric acid concentration

The effects of GABA concentration on the uptake of ^{3}H-GABA were examined by incubating retinae in media containing ^{3}H-GABA (5×10^{-8}M) and various amounts of non-radioactive GABA to give final GABA concentrations ranging from 10^{-6}M to 10^{-4}M. Preliminary experiments showed that the uptake of ^{3}H-GABA was linear over this concentration range for 5 minutes. Therefore, the values obtained after 5 min incubations were used to obtain approximate estimates of the rate of GABA uptake at the various GABA concentrations. These values were found to lie on a straight line when plotted in the form of a linear transformation of the Michaelis–Menton equation (Fig. 3), indicating that GABA uptake in the retina is mediated by a saturable process. The apparent Km for GABA uptake at 25° C is $4{\cdot}0 \times 10^{-5}$M and V_{max}=0·167 (μmol/min)/g retina.

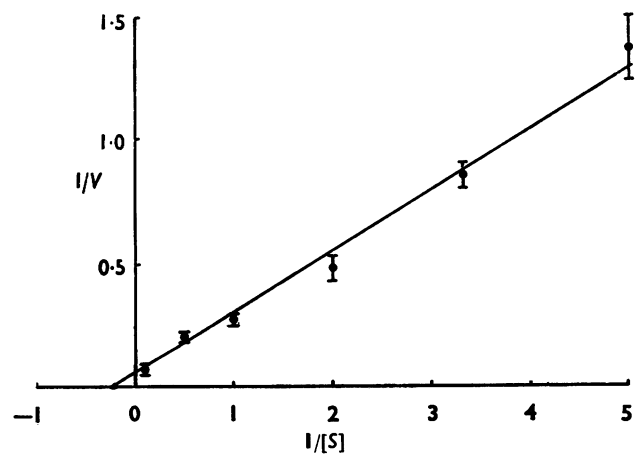

FIG. 3. Kinetic analysis of effect of γ-aminobutyric acid (GABA) concentration on rate of ^{3}H-GABA uptake. V=rate of GABA uptake (10^{-7} moles/min)/g retina, S=GABA concentration (10^{-5}M). Each value is the mean of at least six experiments. Vertical bars indicate S.E.M.

Effect of sodium ion concentration

Retinae were incubated for 10 min in media in which tris/hydrochloric acid buffer, pH 7·4 (50 mM), was substituted for the usual bicarbonate buffer. Varying proportions of the normal sodium chloride content were replaced by choline chloride

or sucrose. The uptake of ^{3}H-GABA by the retina was dependent on sodium ions in the incubation medium (Fig. 4), and in sodium-free medium, the uptake of ^{3}H-GABA was reduced to only 6% of the control values.

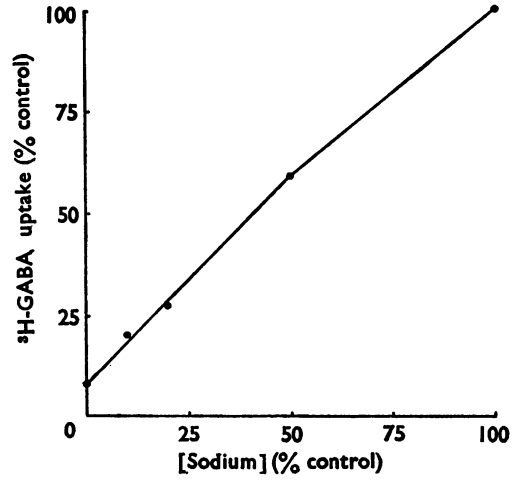

FIG. 4. Effect of sodium ion concentration of ^{3}H-γ-aminobutyric acid (GABA) uptake in retinae incubated for 10 min with ^{3}H-GABA (5×10^{-8}M) in media in which various proportions of normal sodium content were replaced with tris buffer and choline chloride. Each point is the mean of four experiments.

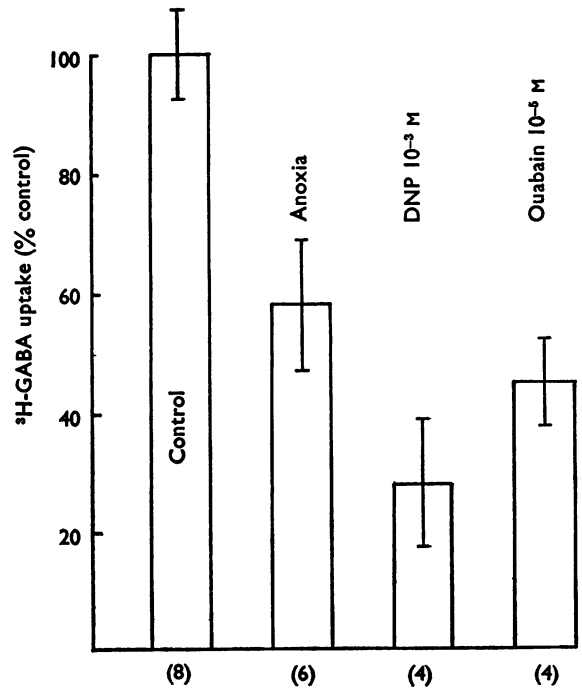

FIG. 5. Effect of preincubation and incubation with metabolic inhibitors, and anoxia, on ^{3}H-γ-aminobutyric acid (GABA) uptake in retinae incubated at 25° C with ^{3}H-GABA (10^{-5}M) for 10 minutes. Figures in parentheses indicate number of experiments, vertical bars indicate S.E.M. DNP=2,4-dinitrophenol.

Effects of metabolic inhibitors

Retinae were preincubated for 15 min in media containing the inhibitor. ^{3}H-GABA and non-radioactive GABA were then added to give a final concentration of 10^{-5}M and the incubations were continued for 10 minutes. The effect of anoxic conditions on the uptake of ^{3}H-GABA by retinae incubated in tris/hydrochloric acid buffer was determined by gassing the media and the flasks with nitrogen. These anoxic conditions produced a small but significant reduction in the uptake of ^{3}H-GABA. The presence of ouabain (10^{-5}M) or 2,4-dinitrophenol (10^{-3}M) caused a large reduction in ^{3}H-GABA uptake (Fig. 5).

Effects of amino acids and analogues of γ-aminobutyric acid

Retinae were given a preliminary incubation for 15 min, then the incubations were continued for 10 min after the addition of ^{3}H-GABA and various amino acids or analogues of GABA. The uptake of ^{3}H-GABA (5×10^{-8}M) was not significantly affected by the presence of glycine, L-glutamate, L-aspartate, L-alanine, L-proline, or L-histidine, at concentrations of 10^{-3}M (Table 1). The analogues of GABA; DL-γ-amino-β-hydroxybutyric acid, β-guanidinopropionic acid and L-2,4-diaminobutyric acid inhibited the uptake of ^{3}H-GABA by 50% at concentrations of 60–80 μM (Table 1).

TABLE 1. *Effect of amino acids and analogues of γ-aminobutyric acid (GABA) on ^{3}H-GABA uptake*

	^{3}H-GABA uptake as % control
Control	100±6·0
Glycine	99±4·2
L-Alanine	109±7·7
L-Histidine	91±5·7
L-Proline	88±9·0
L-Aspartate	107±6·5
L-Glutamate	95±3·0
DL-2-Aminobutyrate	94±6·1
L-2,4,Diaminobutyrate (58 μM)*	50
β-Guanidinopropionate (63 μM)*	50
DL-γ-Amino-β-hydroxybutyrate (80 μM)*	50

^{3}H-GABA uptake was measured in retinae after a 10 min incubation at 25° C with ^{3}H-GABA (5×10^{-8} M) in the presence of various amino acids (10^{-3} M). Results are the mean values ± S.E.M. of four experiments. * The figures in parentheses indicate the concentration of GABA analogue which inhibited the uptake of ^{3}H-GABA by 50% and were obtained by plotting on probability paper: % inhibition of GABA uptake against log concentration of analogue.

Comparison of uptake of ^{3}H-γ-aminobutyric acid by retina and other tissues

Small pieces of cornea and posterior wall of the eye (sclera and choroid) were dissected and weighed. The tissue (approximately 10 mg) was sliced freehand with a razor blade and given a preliminary incubation for 15 min at 25° C. The incubation was then continued for 10 min after the addition of ^{3}H-GABA (5×10^{-7}M). Similar incubations were performed with retinae (10 mg) and small slices of cerebral cortex and liver prepared as described by Iversen & Neal (1968). High tissue:medium ratios were achieved by slices of cerebral cortex and retinae

(Fig. 6) but slices of the posterior wall of the eye, cornea, and liver achieved tissue: medium ratios only in the order of 0·8 to 1·9 (Fig. 6).

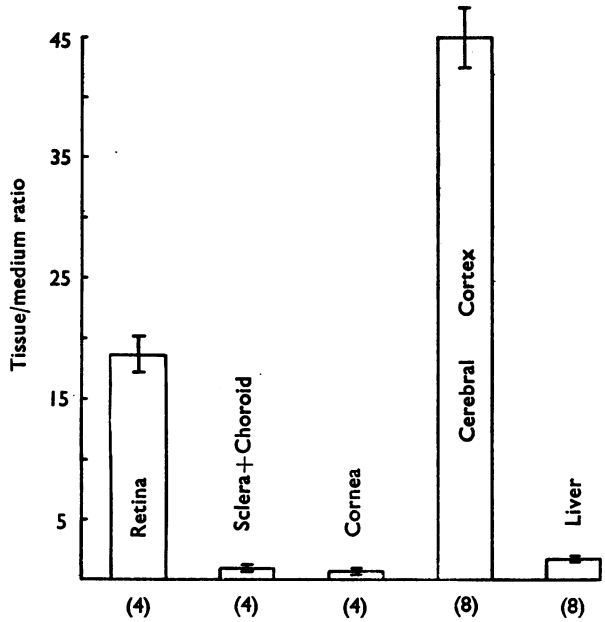

FIG. 6. Uptake of ^{3}H-γ-aminobutyric acid (GABA) by different tissues incubated with ^{3}H-GABA (5×10^{-7}M) for 10 min at 25° C. Figures in parentheses indicate number of experiments; vertical bars indicate S.E.M.

Net uptake of γ-aminobutyric acid by retinae

To establish whether the GABA uptake process could lead to a net increase in the total GABA content of the tissue, retinae were incubated for 30 min in a medium containing a high concentration of ^{3}H-GABA (5×10^{-4}M). At the end of the incubation, the retinae were extracted with acid and assayed for both total GABA and ^{3}H-GABA. The results (Table 2) indicate that a considerable net

TABLE 2. *Net uptake of γ-aminobutyric acid (GABA) by retina*

Sample	^{3}H-GABA	Total GABA	Net uptake of GABA	^{3}H-GABA uptake in excess of net uptake
Control		3·60±0·54		
Experimental	6·62±1·14	9·30±0·29	5·69	0·93

Retinae (20 mg wet weight) were incubated with or without (control) ^{3}H-GABA (0·5 mM) for 30 min at 25° C. The retinae were assayed for total GABA with an amino acid analyser and for ^{3}H-GABA by liquid scintillation counting. Results are mean values ± S.E.M. of four experiments.

uptake of GABA can occur; thus, the total GABA content of the tissue at the end of the incubation was approximately three times higher than that of retinae incubated for the same time in GABA-free medium. The uptake of ^{3}H-GABA was higher than the net increase in tissue GABA content, indicating that some of the ^{3}H-GABA enters the retinae by exchanging with a part of the endogenous GABA. These results show that at the end of a 30 min incubation an average of 25% of

the endogenous pool had exchanged in this way. The uptake of GABA by the retinae did not significantly alter the tissue levels of aspartate, glutamate, glycine or alanine when compared with the levels of these amino acids in retinae incubated in the absence of GABA.

Efflux of ^{3}H-γ-aminobutyric acid from retina

Retinae were incubated with ^{3}H-GABA for 30 min at 25° C in the presence or absence of amino-oxyacetic acid (0·1 mM) and then recovered. The retinae were washed and transferred to fresh medium and the incubations were continued in the absence of GABA. The efflux of radioactivity from the tissue was estimated by sampling the medium at various times. At the end of the efflux experiment, the retinae were recovered in order to determine the amount of radioactivity remaining in the tissue.

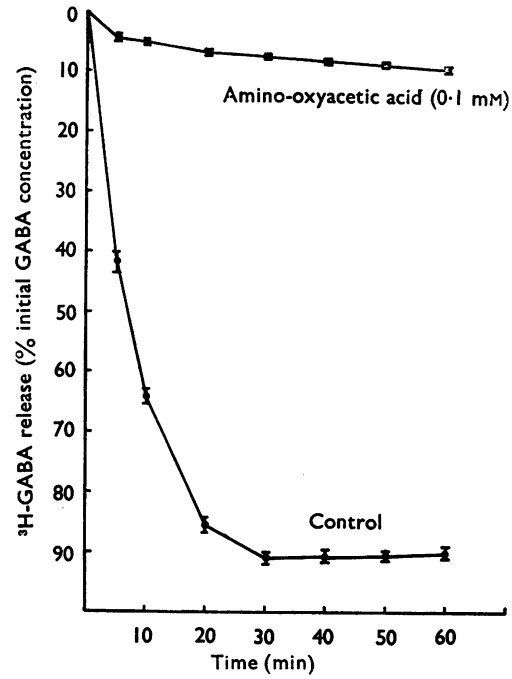

FIG. 7. Efflux of ^{3}H-γ-aminobutyric acid (GABA) from retina. Retinae were incubated with ^{3}H-GABA (5×10^{-8}M) for 30 min at 25° C in the presence (upper trace) or absence (lower trace) of amino-oxyacetic acid (AOAA) (0·1 mM) as appropriate. The release of radioactivity from the retinae into the medium is expressed as a percentage of the ^{3}H-GABA taken up into the tissue during the 30 min incubation. Each value is the mean ±S.E.M. of four experiments.

The efflux of radioactivity from the retinae in the presence or absence of amino-oxyacetic acid is shown in Figure 7. In the absence of amino-oxyacetic acid there was an initial rapid efflux (0–10 min) during which about 65% of the radioactivity was lost from the tissue. This was followed by a slower phase of release (10–30 min), and after a total of 30 min incubation, approximately 90% of the radioactivity was lost from the tissue ; however, in the following 30 min no further radioactivity accumulated in the medium. This pattern of release was strikingly altered

when amino-oxyacetic acid (0·1 mM) was present in the medium used during the incubation of the tissue with ^{3}H-GABA, and in the medium used in the subsequent efflux experiments. In the presence of this inhibitor of GABA-T, there was a rapid release of only about 5% of the radioactivity in the tissue (0–5 min) and in the remaining 55 min only a further 5% of the radioactivity was released.

These results suggested that the much larger efflux of radioactivity from retinae in which GABA-T had not been inhibited, might be due to metabolism of the GABA to products which rapidly diffused from the tissue. This was confirmed by subjecting the media, on completion of the efflux experiment, to ion-exchange chromatography. In the absence of amino-oxyacetic acid, only 22% of the radioactivity released from the tissue was present as ^{3}H-GABA and most of the radioactivity (77%) occurred as ^{3}H-acidic and neutral compounds (Table 3). In contrast, the radioactivity released from retinae exposed to amino-oxyacetic acid was largely ^{3}H-GABA (approximately 70%). The rapid release of radioactive metabolites of GABA, suggested that the measurements of ^{3}H-GABA uptake described, might be underestimates. This is supported by the results (Table 3) which show that the uptake of ^{3}H-GABA by the retina is significantly greater in the presence of amino-oxyacetic acid.

TABLE 3. *Efflux and metabolism of ^{3}H-γ-aminobutyric acid (GABA)*

	Normal medium	Medium containing AOAA (0·1 mM)
Total radioactivity taken up by retina (10 mg) during incubation with ^{3}H-GABA (10^{-7} M)	355±38 nCi	*516±51 nCi
Total radioactivity released from tissue during 60 min incubation in fresh medium	295±24·2 nCi	* 46±6·2 nCi
	Metabolites released as % of total radioactivity released	
^{3}H-GABA (+^{3}H-glutamine)	22·1±0·76	*69·1±1·29
^{3}H-Acidic and neutral compounds	77·9±0·76	*30·9±1·29
^{3}H-Acidic amino acids	≃1·0	Nil

Retinae were incubated with ^{3}H-GABA (10^{-7} M) in media, with or without amino-oxyacetic acid (AOAA) (0·1 mM). They were then incubated in fresh media in the absence of ^{3}H-GABA for 60 min and the radioactivity released was assayed for ^{3}H-GABA and its metabolites by ion-exchange chromatography (Otsuka *et al.*, 1966). The results are the mean ± S.E.M. of four experiments. * Indicates that results with normal medium and medium containing amino-oxyacetic acid were significantly different ($P<0·05$).

Discussion

The present results show that the rat retina possesses the ability to concentrate GABA from an external medium and also confirms other reports that the mammalian central nervous system possesses an uptake mechanism for this amino acid (Elliott & Van Gelder, 1958; Tsukada, Nagata, Hirano & Matsutani, 1963; Blasberg & Lajtha, 1965; Weinstein, Varon, Muhleman & Roberts, 1965; Nakamura & Nagayama, 1966; Iversen & Neal, 1968; Iversen & Johnston, 1971).

Under the conditions used in the present experiments (low concentration of GABA, 25° C, large volume of incubation medium compared with tissue), the retina shows an appreciable ability to concentrate GABA, giving rise to tissue: medium ratios of about 25:1 after an incubation of 30 minutes. Under these conditions practically all the radioactivity in the tissue was present as ^{3}H-GABA. The

process responsible for the accumulation of GABA shows many of the properties characteristic of an active uptake system; thus GABA is accumulated against a considerable concentration gradient, the uptake is saturable, temperature-sensitive, sodium-dependent and inhibited by anoxia, dinitrophenol and ouabain.

It is unlikely that the uptake of ^{3}H-GABA is simply due to exchange diffusion with the large endogenous pool of GABA (1–3 μmol/g); such an exchange process would not be expected to exhibit the properties described above, nor could it account for GABA uptake leading to a large net increase in the tissue GABA content. It seems likely, therefore, that the accumulation of ^{3}H-GABA represents a unidirectional influx mediated by an active transport mechanism.

This suggestion is supported by the small efflux of ^{3}H-GABA which was found to occur when retinae, which had been previously incubated with ^{3}H-GABA in the presence of amino-oxyacetic acid, were placed in fresh medium containing no GABA; under these conditions, where GABA-T was inhibited, only 10% of radioactivity was lost from the tissue during a 60 min incubation. However, when similar efflux experiments were performed in the absence of amino-oxyacetic acid, 90% of radioactivity was lost from the tissue, largely as ^{3}H-acid metabolites. These results suggest that the tissue:medium ratios for ^{3}H-GABA obtained in the present experiments are probably underestimates, especially at longer incubation times. This has been confirmed in the present study and in experiments in which the uptake of ^{3}H-GABA has been measured in the presence or absence of amino-oxyacetic acid. Tissue:medium ratios after 10 min were not significantly different in the two groups, but after 60 min, the tissue incubated in the presence of the inhibitor of GABA-T had accumulated approximately twice as much radioactivity as the controls (Neal & Starr, 1973). These results are consistent with the suggestion that GABA is accumulated by an essentially unidirectional process, but following uptake, a substantial proportion of the ^{3}H-GABA is eventually metabolized, largely to ^{3}H-acid metabolites, which are then rapidly lost from the tissue. Such a mechanism might explain why the uptake of ^{3}H-GABA by the retina is greater at 25° C than at 37° C, since at the higher temperature a larger proportion of the ^{3}H-GABA would be metabolized and subsequently lost from the tissue.

The uptake of GABA by brain tissue is mediated by a process with remarkable specificity (Blasberg & Lajtha, 1965; Iversen & Neal, 1968; Iversen & Johnston, 1971). The present studies confirm that GABA uptake in the central nervous system is unaffected by large molar excesses of related amino acids such as glycine, alanine, glutamate, aspartate or histidine. The more closely related compounds, β-guanidinopropionic acid and 2,4-diaminobutyric acid effectively inhibited GABA uptake at 0·1 mM. A similar pattern of specificity was found in previous studies of GABA uptake in small slices of rat cerebral cortex (Iversen & Neal, 1968).

The precise cellular sites of uptake of ^{3}H-GABA by the central nervous system are not known. Recent autoradiographic studies of the distribution of ^{3}H-GABA in rat brain slices *in vitro* (Hökfelt & Ljungdahl, 1970; Bloom & Iversen, 1971) and in the retina *in vitro* and *in vivo* (Ehinger, 1970; Lam & Steinman, 1971; Ehinger & Falck, 1971; Neal & Iversen, 1972), suggest that the labelled amino acid is selectively accumulated by certain nerve-endings, neurones, and neuroglia. When rat retinae were incubated with ^{3}H-GABA under the conditions of the present study, a large proportion of the radioactive amino acid was found to be taken up and accumulated by the neuroglial Müller cells; a smaller proportion of the ^{3}H-GABA

was accumulated by cells in the inner nuclear layer which had the position of amacrine cells (Iversen & Neal, 1972). Thus, it seems likely that in the rat retina ^{3}H-GABA is accumulated by both neuroglia and a relatively small number of neurones.

The uptake sites for GABA appear to occur almost exclusively in central nervous tissue. Slices of cornea or of the posterior wall of the eye, or liver, failed to show any ability to concentrate ^{3}H-GABA. Recently, sympathetic ganglia have been shown to accumulate ^{3}H-GABA *in vitro* and the uptake process appears to have properties similar to that in retina and cerebral cortex (Bowery & Brown, 1971). However, the uptake of ^{3}H-GABA into ganglia is extremely slow, presumably due to a low concentration of uptake sites in this tissue compared with central nervous tissue.

The uptake process for GABA in the retina was remarkably similar to that described previously in cerebral cortex (Iversen & Neal, 1968). In both tissues the amino acid is transported by an uptake process with a high affinity, the apparent Km for retina and cortex being 40 μM and 22 μM respectively. This uptake process would provide an effective mechanism for terminating the action of GABA on neurones if it is released from inhibitory nerve endings. Similar high affinity uptake processes exist in the central nervous system for the other putative amino acid transmitters; glutamate, aspartate and glycine (Neal & Pickles, 1969; Johnston & Iversen, 1971; Neal, 1971; Logan & Snyder, 1971). Noradrenaline is also thought to be inactivated by a reuptake process, and after its release, following presynaptic nerve stimulation, it appears to be taken up largely by adrenergic nerve terminals (Iversen, 1967). However, if GABA is released from inhibitory nerve endings in the rat retina, then it would presumably be inactivated largely by uptake into the neuroglial Müller cells. Uptake of GABA by Müller cells has also been suggested by Graham (1972) to explain why GABA is more evenly distributed across the layers of the retina than glutamate decarboxylase, the enzyme responsible for GABA formation.

The suggestion that amino acids such as GABA might be taken up by neuroglia is in keeping with an early suggestion that these cells remove and inactivate deleterious products of neuronal activity (Lugaro, 1907).

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