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EFFECTS OF CHANGES IN THE IONIC COMPOSITION OF THE INCUBATION MEDIUM ON THE ACCUMULATION AND METABOLISM OF ^{3}H-γ-AMINOBUTYRIC ACID AND ^{14}C-TAURINE IN ISOLATED RAT RETINA

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Abstract—The uptake of ^{14}C-taurine and ^{3}H-γ-aminobutyric acid (^{3}H-GABA) after 10 min and the accumulation of these amino acids after 60 min by retinae incubated in media of varying ionic composition have been studied. The presence of ^{3}H-GABA did not modify the uptake of ^{14}C-taurine, and vice versa. Sodium was found to be indispensable for the uptakes of both amino acids. Raised concentrations of sodium increased both the uptake and accumulation of ^{14}C-taurine and ^{3}H-GABA, reaching a maximum at 196 mM Na^{+}. The amounts taken up for both were lower in the absence of potassium. High levels of this ion (60 mM) reduced both the uptake and the subsequent accumulation of ^{14}C-taurine. However, at this concentration, potassium did not affect ^{3}H-GABA uptake after 10 min, but increased the amount of ^{3}H-GABA accumulated after 60 min. Higher concentrations (80–100 mM) caused a reduction in this accumulation. Aminooxyacetic acid (AOAA) potentiated the accumulation of ^{3}H-GABA from control medium but suppressed accumulation from high sodium or potassium (60 mM) media. In the latter media, the tissue content of acidic ^{3}H-metabolites was markedly lower than in control retinae. The interpretations of these findings are discussed with reference to the possible actions of these ions on the efflux of ^{3}H-GABA and its metabolites from retina.

In calcium-free medium ^{14}C-taurine uptake was reduced but ^{3}H-GABA uptake was not. At longer periods of incubation the accumulation of both amino acids was increased under these conditions. Magnesium and chloride ions did not affect the uptake or accumulation of either amino acid.

BOTH γ-aminobutyric acid (GABA) and taurine have been shown to be capable of inhibiting the electrical activity of retinal[1,16] or cortical[2] neurones. Also, both of these amino acids have been found to occur in a free form in the retina,[11,13] and may therefore be considered to be putative natural inhibitory neurotransmitters or modulators in this tissue.

Some investigators believe that one of the pre-requisites for such a transmitter role is that the tissue should possess a specific high affinity uptake system capable of terminating the actions of the compound released at the synapse.[5,6] Neal *et al.*[9] demonstrated recently the existence of transport systems with a high affinity for taurine and GABA in rat retina. These mechanisms are sodium-dependent and are sensitive to metabolic inhibitors. A detailed study of the ionic requirements for the uptake of these amino acids in the retina has not been reported. Therefore, in the present experiments, the effects of altering the concentrations of several ions in the incubation medium on the uptake of taurine and GABA by isolated rat retina have been studied.

MATERIALS AND METHODS

General experimental procedure. Wistar albino rats of either sex and weighing 120–200 g were used. The animals were killed by cervical dislocation, the eyes enucleated and the retinae quickly dissected out in ice-cold medium. The retinae were preincubated for 15 min at 37°, then ^{14}C-taurine (2×10^{-5} M, 0·05 μCi/ml) and/or ^{3}H-GABA (2×10^{-5} M, 0·05 μCi/ml) were added and the incubation continued for 10 or 60 min. The tissues were then recovered and washed in fresh medium, dried in an oven at 56°, weighed and dissolved in 0·5 ml soluene Tm 100 (Packard). Afterwards, the solutions were brought to pH 7 by the dropwise addition of glacial-acetic acid, followed by 4 ml ethoxyethanol and 10 ml 1% butyl PBD in toluene, and the radioactivity measured in a liquid scintillation counter (Packard model 2003). All counts/min were corrected for background radiation and for quenching. The resulting values for dis/min were then used to calculate the total amounts of amino acid accumulated during the 10 min or 60 min incubation periods, which were expressed as micromoles of amino acid/gram of dry weight of retina.

Metabolism of ^{3}H-GABA. Retinae were incubated with ^{3}H-GABA (2×10^{-5} M) for 60 min at 37°. The radioactivity accumulated by the tissues was recovered and the unchanged ^{3}H-GABA and ^{3}H-amino acids separated from acidic ^{3}H-metabolites by ion-exchange chromatography as described in detail previously.[14] Since the amount of ^{3}H-GABA converted into ^{3}H-amino acids by rat retina is very small (less than 5 per cent) it has been neglected for the purposes of the present experiments.

The incubation medium was Krebs bicarbonate solution of the following composition: NaCl, 118 mM; KCl, 4·75 mM; $CaCl_2$, 2·54 mM; KH_2PO_4, 1·2 mM; $MgSO_4$, 1·22 mM; $NaHCO_3$, 24·75 mM; glucose, 5·55 mM; distilled water to 1 litre. The solution was bubbled with 5% carbon dioxide in oxygen to give a final pH of 7·4. In some experiments the composition of the medium was altered by omitting certain ions, but in each case the pH was kept constant by the addition of Tris–HCl buffer (50 mM, pH 7·4), and the tonicity was maintained by the addition of appropriate quantities of choline chloride. In some other experiments the normal medium was supplemented by the further addition of sodium chloride, potassium chloride or choline chloride (controls for the increase in tonicity).

GABA-2,3-^{3}H (2 Ci/mmole, diluted to 4·8 mCi/mmole with non-radioactive GABA) and taurine-1,2-^{14}C (2·5 mCi/mmole) were obtained from New England Nuclear, 6072 Dreieichenhain, West Germany. Aminooxyacetic acid (AOAA) was obtained from Aldrich Chemical Company Inc., Milwaukee, Wisconsin; Soluene Tm 100 from Packard Instrument Company Ltd., 2200 Warrenville, Illinois; butyl PBD from Fisons Scientific Appliances Ltd., Loughborough; toluene, glacial-acetic acid and choline chloride from BDH Chemicals Ltd., Poole.

RESULTS

Earlier studies[3,5,14,15,20] have shown that the uptake of ^{14}C-taurine and ^{3}H-GABA are linear during the first 10 min of incubation but not after longer incubation times, when a balance between uptake, metabolism and efflux of the amino acid is achieved. For the purposes of the present study, therefore, the quantities of amino acid recovered from the tissue after 10 min incubation are referred to as "uptake" and after 60 min incubation as "accumulation".

When retinae were incubated for 10 min at 37° with ^{14}C-taurine or ^{3}H-GABA (2×10^{-5} M), both amino acids were taken up rapidly by the tissue; the uptake of ^{3}H-GABA was approximately one and a half-times greater than that for ^{14}C-taurine (Table 1). Similarly, the accumulation of ^{3}H-GABA after 60 min incubation was roughly one and a half times higher than the accumulation of ^{14}C-taurine over this period. The addition of non-radioactive GABA (2×10^{-5} M) to the incubation medium did not apparently interfere with the quantity of ^{14}C-taurine taken up by the tissue, either at 10 or 60 min incubations. Also, the inclusion of non-radioactive taurine (2×10^{-5} M) in the medium was without effect on the accumulation of ^{3}H-GABA at these times (Table 1). In subsequent experiments, therefore, the uptake (after 10 min) or the accumulation (after 60 min) of both amino acids were studied together in the same tissue samples.

TABLE 1. CONTROL UPTAKE AND ACCUMULATION OF ^{14}C-TAURINE AND ^{3}H-GABA

Amino acid	Concn in medium (M)	Amino acid in tissue, concn (μmole/g dry wt) after incubation for: 10 min ^{14}C-Taurine	10 min ^{3}H-GABA	60 min ^{14}C-Taurine	60 min ^{3}H-GABA
^{14}C-Taurine	2×10^{-5}	0·60 ± 0·02		2·80 ± 0·17	
^{14}C-Taurine + cold GABA	2×10^{-5} + 2×10^{-5}	0·56 ± 0·02		2·75 ± 0·18	
^{3}H-GABA	2×10^{-5}		0·84 ± 0·02		4·32 ± 0·23
^{3}H-GABA + cold taurine	2×10^{-5} + 2×10^{-5}		0·81 ± 0·02		4·34 ± 0·22

Retinae were preincubated for 15 min at 37°, then radioactive amino acid (± cold amino acid as appropriate) was added and the incubation continued for a further 10 min (to determine uptake) or 60 min (to determine accumulation). The retinae were then washed, dried at 56° and weighed. The dried tissues were dissolved in 0·5 ml soluene, 4 ml ethoxyethanol and 10 ml 1% butyl PBD in toluene added, and radioactivity measured in a liquid scintillation counter. Each value is the mean ± S.E.M. of at least eight experiments, and is expressed as the total amount of radioactive amino acid recovered per unit dry weight of retina.

Effect of sodium ions. The total sodium ion concentration of the normal medium was 142 mM. When the sodium salts were replaced with an equivalent concentration of choline chloride, both the uptake and accumulation of ^{14}C-taurine and ^{3}H-GABA by retinae after 60 min incubation in this medium were almost completely abolished ($P < 0·001$, Table 2). By contrast, retinae maintained in media containing levels of sodium above normal accumulated correspondingly greater amounts of both amino acids. The optimum sodium level producing this effect was found to be 196 mM ($P < 0·001$); at higher levels the accumulation of both compounds declined.

Studies of the uptakes of ^{14}C-taurine and ^{3}H-GABA at an elevated sodium concentration (196 mM) revealed that these, too, were significantly increased ($P < 0·001$, Table 2). These changes are almost certainly not attributable to the increase in chloride ion content of the medium, since no such differences in either the uptake or the accumulation of these amino acids were detected when the excess sodium chloride was replaced by choline chloride (54 mM).

TABLE 2. EFFECT OF SODIUM IONS

Concn sodium in medium (mM)	Amino acid in tissue, concn (μmole/g dry wt) after incubation for: 10 min		60 min	
	^{14}C-Taurine	^{3}H-GABA	^{14}C-Taurine	^{3}H-GABA
0	0·12 ± 0·01	0·08 ± 0·01	0·15 ± 0·01	0·37 ± 0·02
142*	0·62 ± 0·02	0·83 ± 0·02	2·78 ± 0·13	4·33 ± 0·13
169			4·78 ± 0·23	5·76 ± 0·18
182			4·88 ± 0·23	6·15 ± 0·25
196	0·90 ± 0·03	1·03 ± 0·02	5·03 ± 0·24	6·41 ± 0·27
210			4·77 ± 0·18	6·13 ± 0·26
223			4·25 ± 0·17	5·81 ± 0·27

* Concentration of sodium ions present in normal medium.
For experimental details see legend to Table 1. Each value is the mean ± S.E.M. of at least eight experiments.

Effect of potassium ions. Similar experiments were performed in which the potassium ion content of the normal medium (5·95 mM) was varied (Table 3). In the complete absence of this cation, and when the tonicity and the pH of the medium were kept constant, both the uptake and the accumulation of ^{14}C-taurine and ^{3}H-GABA were significantly lowered ($P < 0·005$), although to a much smaller extent than by the omission of sodium. By contrast, increasing the potassium concentration in the medium affected the retinal uptake and accumulation of these amino acids in different ways. Thus the accumulation of ^{14}C-taurine after 60 min steadily decreased as the potassium concentration was increased to 100 mM. With 60 mM potassium, the uptake of ^{14}C-taurine was also found to be suppressed compared with its uptake (controls) in normal medium ($P < 0·001$). On the other hand, the uptake of ^{14}C-taurine from a medium containing choline chloride (54 mM) instead of the excess potassium chloride did not differ significantly from controls.

TABLE 3. EFFECT OF POTASSIUM IONS

Concn potassium in medium (mM)	Amino acid in tissue, concn (μmole/g dry wt) after incubation for: 10 min		60 min	
	^{14}C-Taurine	^{3}H-GABA	^{14}C-Taurine	^{3}H-GABA
0	0·48 ± 0·02	0·54 ± 0·01	2·13 ± 0·08	1·83 ± 0·22
5·95*	0·62 ± 0·02	0·83 ± 0·02	2·78 ± 0·04	4·33 ± 0·08
20			2·28 ± 0·13	4·76 ± 0·05
40			1·64 ± 0·12	5·21 ± 0·08
60	0·29 ± 0·01	0·75 ± 0·02	1·38 ± 0·09	5·30 ± 0·09
80			1·02 ± 0·04	4·06 ± 0.08
100			1·00 ± 0·02	3·62 ± 0·03

* Concentration of potassium ions present in normal medium.
Experimental details as for Table 1. Each value is the mean ± S.E.M. of at least eight experiments.

Unlike ^{14}C-taurine, the accumulation of ^{3}H-GABA was increased as the potassium concentration was raised to 60 mM ($P < 0.001$), although the initial rate of uptake of ^{3}H-GABA remained unchanged at this concentration (Table 3). At higher potassium concentrations (80–100 mM) the opposite effect was observed and the accumulation of ^{3}H-GABA was reduced to below control levels. As with ^{14}C-taurine, however, neither the uptake nor the accumulation of ^{3}H-GABA in the presence of choline chloride (54 mM) differed significantly from control values.

TABLE 4. EFFECTS OF CALCIUM AND MAGNESIUM IONS

Ion	Concn in medium (mM)	Amino acid in tissue, concn. (μmole/g dry wt) after incubation for: 10 min ^{14}C-Taurine	10 min ^{3}H-GABA	60 min ^{14}C-Taurine	60 min ^{3}H-GABA
Calcium Magnesium	2·54 1·22 *	0·62 ± 0·02	0·83 ± 0·02	2·78 ± 0·04	4·33 ± 0·08
Calcium Magnesium	0 1·22	0·48 ± 0·03	0·88 ± 0·02	3·87 ± 0·14	5·17 ± 0·18
Calcium Magnesium	2·54 0	0·60 ± 0·01	0·84 ± 0·01	2·82 ± 0·01	4·41 ± 0·04

* Concentrations of these ions present in normal medium.
Experimental details as for Table 1. Each value is the mean ± S.E.M. of at least eight experiments.

Effects of magnesium and calcium ions. When retinae were incubated in media containing choline chloride in place of the magnesium salts no significant differences in either the uptake or the accumulation of ^{14}C-taurine or ^{3}H-GABA could be detected. The results are shown in Table 4. On the other hand, the similar replacement of calcium ions caused a reduction in the initial uptake of ^{14}C-taurine ($P < 0.005$) but not that of ^{3}H-GABA, while after 60 min incubation the accumulation of both ^{14}C-taurine ($P < 0.001$) and ^{3}H-GABA ($P < 0.005$) were significantly increased.

Effect of AOAA. One possible way that changing the ionic composition of the medium may affect the retinal accumulation of these amino acids during prolonged periods of incubation is by modifying the rates at which they are metabolized by the tissue. Table 5 illustrates the effects of AOAA on the accumulation of ^{14}C-taurine and ^{3}H-GABA at a concentration (10^{-5} M) which has been demonstrated to inhibit completely the metabolism of GABA but not that of taurine.[10]

Under no circumstances was the accumulation of ^{14}C-taurine altered by AOAA, whether the incubation was carried out in normal, high sodium (196 mM) or high potassium (60 mM) medium. By contrast, ^{3}H-GABA accumulation was significantly potentiated by AOAA in normal medium ($P < 0.001$;[10]). More surprisingly, however, previous exposure to AOAA caused a significant reduction in the accumulation of this amino acid from the high sodium (196 mM) and also the high potassium (60 mM) medium ($P < 0.005$).

Metabolism of ^{3}H-GABA. Retinae were incubated in normal, high sodium (196 mM) or high potassium (60 mM) medium for 60 min at 37° in order to determine whether

TABLE 5. EFFECT OF AOAA

Medium	Concn of amino acid in tissue after incubation for 60 min (μmole/g dry wt)			
	^{14}C-Taurine		^{3}H-GABA	
	Controls	+AOAA	Controls	+AOAA
Normal	2·47 ± 0·23	2·40 ± 0·06	4·16 ± 0·08	7·15 ± 0·11
High sodium (196 mM)	4·84 ± 0·14	4·88 ± 0·15	6·01 ± 0·17	4·63 ± 0·12
High potassium (60 mM)	1·22 ± 0·05	1·20 ± 0·04	5·27 ± 0·14	4·20 ± 0·04

Where AOAA (10^{-5} M) was included in the medium it was present throughout the preincubation and incubation periods. Experimental details are otherwise the same as for Table 1. Each value is the mean ± S.E.M. of at least eight experiments.

high concentrations of these ions could affect the tissue levels of acidic ^{3}H-metabolites accumulated during this period. The results are shown in Table 6. On average, the normal tissue levels of these metabolites amounted to 22·4 per cent of the total radioactivity extracted from the tissue. However, no appreciable quantities of these metabolites could be detected in retinae which had been incubated for this time in the high sodium or high potassium media.

TABLE 6. METABOLISM OF ^{3}H-GABA

Medium	Concn of ^{3}H-metabolites in tissue after 60 min incubation (% total tissue radioactivity)
Normal	22·4 ± 3·7
High sodium (196 mM)	0
High potassium (60 mM)	0

Retinae were incubated with ^{3}H-GABA (2×10^{-5} M, 0·05 μCi/ml) in the above media for 60 min at 37°. The tissues were then washed in fresh medium, homogenized, and the radioactivity separated by ion-exchange chromatography on Amberlite CG-120 resin (H^+ form, 200 mesh) into ^{3}H-amino acids and acidic ^{3}H-metabolites as described in detail elsewhere.[14] Each value is the mean ±S.E.M. of six experiments.

DISCUSSION

In the present experiments an attempt has been made to distinguish between the actions of various ions on the uptake of taurine and GABA by retina after a short period of incubation (10 min), and their accumulation by this tissue at longer incubation times (60 min), the latter presumably reflecting a balance of uptake, metabolism and release of the compounds. As demonstrated in previous studies, sodium ions are essential for the uptake of taurine and GABA in central nervous tissue.[5,8,14,18,19,21] The present data confirm these findings and indicate that sodium is also necessary for the long-term accumulation of these two amino acids. The results also show that

potassium and calcium can strongly influence these processes in retina, whereas magnesium and chloride ions are without effect on these parameters.

According to the model proposed by Weinstein *et al.*,[21] the sodium-dependence of the transport mechanisms for these two amino acids in retina suggests that the inward movement of both taurine and GABA into retinal cells is coupled to the passage of sodium down its electrochemical gradient. The correct sodium balance may be maintained by the sodium–potassium-dependent ATPase acting as a sodium pump. This model could explain why the uptakes of taurine and GABA are both potassium-sensitive, and also why both uptakes are increased when the external sodium concentration is raised above normal. In this connection it is interesting to note that the optimum concentration of sodium producing this effect in retina (196 mM) is practically identical with that found previously using brain cortex slices (200 mM).[12]

On the other hand, it is possible that the suppression of taurine uptake by high potassium levels reflects an increase in the rate of efflux of this amino acid, which may occur as a result of the tissue being depolarised by this ion.[4,7] If this is true, then it is surprising that GABA uptake is not similarly affected, especially as it has already been demonstrated that the rate of efflux of GABA from retina is accelerated by potassium at this concentration.[20] A possible explanation for this discrepancy is that GABA turnover may be slowed down under the influence of potassium, as in brain slices,[7] such that the consequent increase in the tissue content of accumulated GABA compensates for any concomitant increase in its efflux. Nevertheless, the predominant action of potassium at higher concentrations (80–100 mM) almost certainly appears to be on the efflux of GABA rather than on its metabolism, since the overall accumulation of GABA under these conditions is considerably lower than with 60 mM potassium.

The reason why AOAA inhibits the potentiation of accumulation of GABA in retinae incubated in high sodium or high potassium media is not immediately obvious from the present data. Neal and Starr[10] have shown that AOAA (10^{-5} M) inhibits completely the activity of the enzyme 2-oxoglutarate-4-aminobutyrate aminotransferase in the retina, which in turn leads to an increase in the amount of exogenous GABA accumulated. If the metabolism of GABA is similarly reduced by high levels of sodium or potassium ions, as Table 6 would appear to indicate, then AOAA would not be expected to influence the accumulation of GABA by tissues exposed to high concentrations of these ions. However, the possibility that these ions may increase the rate of elimination of GABA metabolites from the tissue cannot be ruled out, and is currently being investigated.

Finally the role of calcium in the retinal transport of these amino acids appears to be equally complex. Thus while GABA uptake is not altered in the absence of calcium,[17] the uptake of taurine is significantly reduced under these conditions ($P < 0{\cdot}005$). The reason for this difference in behaviour towards calcium at short incubation periods is not understood, especially in view of the fact that the accumulation of both amino acids is increased following their incubation with the tissues for 60 min. However, it is not known whether calcium is important for the efflux of these amino acids from retina, and this possibility is therefore being studied.

In conclusion, the present findings emphasize the importance of sodium ions in the uptake mechanisms for taurine and GABA in rat retina. They also illustrate the striking differences in the ionic requirements of these two uptake processes in retina, and

hence support the suggestion that these amino acids are transported by separate systems in this tissue.

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REFERENCES

1. A. AMES and D. A. POLLEN, *J. Neurophysiol.* **32,** 424 (1969).
2. D. R. CURTIS and J. C. WATKINS, *J. Neurochem.* **6,** 117 (1960).
3. M. GOODCHILD and M. J. NEAL, *Br. J. Pharmac.*, **47,** 529 (1973).
4. H. H. HILLMAN and H. J. MCILWAIN, *J. Physiol., Lond.* **157,** 263 (1961).
5. L. L. IVERSEN and M. J. NEAL, *J. Neurochem.* **15,** 1141 (1968).
6. W. J. LOGAN and S. H. SNYDER, *Brain Res.* **42,** 413 (1972).
7. Y. MACHIYAMA, R. BALAZS, B. J. HAMMOND, T. JULIAN and D. RICHTER, *Biochem. J.* **116,** 469 (1970).
8. D. L. MARTIN and A. A. SMITH, *J. Neurochem.* **19,** 841 (1972).
9. M. J. NEAL, D. G. PEACOCK and R. D. WHITE, *Br. J. Pharmac.*, **47,** 656P (1973).
10. M. J. NEAL and M. S. STARR, *Br. J. Pharmac.* **47,** 543 (1973).
11. H. PASANTES-MORALES, J. KLETHI, M. LEDIG and P. MANDEL, *Brain Res.* **41,** 494 (1972).
12. K. SANO and E. ROBERTS, *Biochem. Pharmac.* **12,** 489 (1963).
13. M. S. STARR, *Brain Res.*, **47,** 543 (1973).
14. M. S. STARR and M. J. VOADEN, *Vision Res.* **12,** 549 (1972).
15. M. S. STARR and M. J. VOADEN, *Vision Res.* **12,** 1261 (1972).
16. M. STRASCHILL and J. PERWEIN, *J. Pflügers Archs.* **312,** 45 (1969).
17. Y. TSUKADA, Y. NAGATA, S. HIRANO and T. MATSUTANI, *J. Neurochem.* **10,** 241 (1972).
18. S. VARON, H. WEINSTEIN, C. F. BAXTER and E. ROBERTS, *Biochem. Pharmac.* **14,** 1755 (1965).
19. S. VARON, H. WEINSTEIN, T. KAKEFUDA and E. ROBERTS, *Biochem. Pharmac.* **14,** 1213 (1965).
20. M. J. VOADEN and M. S. STARR, *Vision Res.* **12,** 559 (1972).
21. H. WEINSTEIN, S. VARON, D. R. MUHLEMAN and E. ROBERTS, *Biochem. Pharmac.* **14,** 273 (1965).