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Subcellular Distribution of Taurine and Cysteinesulphinate Decarboxylase in Developing Rat Brain

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The concentration of taurine and the activities of cysteinesulphinate decarboxylase and glutamate decarboxylase have been measured in rat brain. During development, taurine exhibited a decrease in concentration unrelated to the activity of cysteinesulphinate decarboxylase which increased during the same period. The distribution of taurine in subcellular fractions of adult and 7-day-old rat brain was typical of most amino acids, whereas half of the cysteinesulphinate decarboxylase activity was found in the nerve-ending cytoplasm. In anatomical distribution, taurine displayed great regional heterogeneity but both cysteinesulphinate decarboxylase and glutamate decarboxylase were more evenly distributed. Hypertaurinaemia was shown to have no effect on the entry of glycine into the brain or on its utilization in protein synthesis.

In immature neural tissue glutamate and taurine dominate the free amino acid pool but whereas the concentration of glutamate and related compounds increases during early development that of taurine decreases. Relatively smaller changes in the concentration of other amino acids have been observed (Agrawal, Davis & Himwich, 1966). Increase in concentration of dicarboxylic amino acids and their derivatives (e.g. γ-aminobutyrate) is paralleled by rising activity of such enzymes as glutamate decarboxylase (EC 4.1.1.15) (Sims & Pitts, 1970) and other enzymes concerned in the intermediary metabolism of glutamate.

Since taurine is probably formed through decarboxylation of cysteinesulphinic acid (EC 4.1.1.29) (Peck & Awapara, 1967) we have examined the activity of cysteinesulphinate decarboxylase during development of rat brain in relation to taurine concentration. A further reason for this study is the controversial suggestion that both glutamate and cysteinesulphinate are decarboxylated by the same enzyme in rat brain (Davison, 1956; Jacobsen, Thomas & Smith, 1964).

Whereas the role of glutamate and γ-aminobutyrate in the nervous system has received much attention relatively little is known about the possible function of taurine. The possibility that taurine may have a depressant action on neurones (Curtis & Watkins, 1965) prompted us to examine the localization of taurine and cysteinesulphinate decarboxylase in the brain and its subcellular fractions. A preliminary account of this work has been published (Kaczmarek, Agrawal & Davison, 1970).

METHODS AND MATERIALS

Animals. Albino rats of the Wistar strain bred in our animal colony were used throughout this study. Litters were decreased to six at birth and the constant litter size was maintained until weaning. Young adults (100–120g) of both sexes were used. The rats were killed by decapitation and the brains quickly removed and where appropriate frozen on solid CO_2 or homogenized immediately in 10% (w/v) trichloroacetic acid for the extraction of taurine.

Reagents. In general AnalaR reagents were used. Taurine was obtained from Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K., and L-glutamic acid from Hopkin and Williams Ltd., Chadwell Heath, Essex, U.K. The ion-exchange resins Zeo-Karb 325 and Dowex AG 1 (X10) were purchased from BDH Chemicals Ltd., Poole, Dorset, U.K. and Bio-Rad Laboratories, Richmond, Calif., U.S.A. respectively. [U-^{14}C]Glycine was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. *o*-Phthalaldehyde was from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K.

Enzyme preparation. Fresh brain was homogenized in 0.067M-Sörensen's phosphate (Na_2HPO_4–KH_2PO_4) buffer containing 0.2% (w/v) Triton X-100 and stored at −20°C until assayed for enzyme activity. The extracts were usually analysed for enzyme activity within 24h, for longer storage even at low temperatures leads to some loss of activity. Cysteinesulphinate decarboxylase and glutamate decarboxylase were assayed essentially by the technique of Davison (1956). For enzyme-activity determination in various subcellular fractions, pellets were homogenized in the same 0.067M-phosphate buffer with

Triton X-100 added to 0.2% (w/v) final concentration. Phosphate (Na_2HPO_4–KH_2PO_4) buffer (1 M) was added to the soluble fractions to make the final concentration 0.067 M-phosphate. The enzyme preparation (3 ml) was placed in a Warburg flask and 0.05 ml of pyridoxal phosphate solution (2.5 mg/ml in phosphate buffer) was added. Cysteinesulphinate or glutamate (0.2 ml) was added to give a final concentration of 0.01 M. Control samples in which 0.2 ml of 0.67 M-phosphate buffer solution was added in place of the substrate were also included. The samples were gassed with N_2 for 5–7 min. After a 20–30 min incubation at 37°C, evolution of CO_2 was measured by taking readings every 10 min for up to 1 h. The small evolution of gas from the control sample was subtracted from that with substrate added. The method of least squares was used for calculation of gas evolution (Aldridge, Berry & Davies, 1949).

Subcellular fractionation. Subcellular fractions were prepared from 7-day-old and adult rats essentially by the technique of De Robertis and co-workers (De Robertis, Pellegrino de Iraldi, Rodriguez de Lorez Arnaiz & Salganicoff, 1962; De Robertis, Rodriguez de Lorez Arnaiz, Salganicoff, Pellegrino de Iraldi & Zieher, 1963), with the modification that the brain was homogenized in 0.32 M-sucrose containing 10 μM-Ca^{2+} in a tight-fitting Teflon–glass homogenizer (clearance on diameter 100 μm) with four up-and-down strokes at a speed of 1500 rev./min. For the preparation of the synaptic vesicles fraction (M_2) the concentration of the sucrose in the crude mitochondrial fraction was lowered to 0.06 M (Kuriyama, Roberts & Vos, 1968) instead of 0.032 M as suggested by De Robertis *et al.* (1963). After preparation each fraction was diluted with excess of 0.16 M-sucrose and centrifuged at 100000*g* for 30 min. The supernatant was discarded and pellets were used either for measurement of enzyme activity or taurine assay.

Isolation of free amino acids. Fresh or frozen brains were homogenized in ice-cold 10% (w/v) trichloroacetic acid (6 ml/g of tissue) in an all-glass–Teflon motor-driven homogenizer at 1500 rev./min for 2 min at 0°C. The homogenate was centrifuged at 20000*g* for 45 min in an MSE 50 centrifuge. The clear supernatant was carefully collected in a measuring cylinder by filtration through Whatman no. 54 paper. The residue was extracted with 2 × 5 ml of 10% (w/v) trichloroacetic acid, with centrifugation after each extraction for 30 min. The filtered washings were combined with the original extract. To remove trichloroacetic acid the acid extract was washed four times with 4 vol. of anhydrous ether and made up to a known volume (25 ml) with water.

Ion-exchange separation of taurine from other free amino acids. A portion of the extract (10 ml) was passed through a two-column assembly consisting of a sulphonic resin (Zeo-Karb 325; H^+ form; 1 cm × 10 cm long) fitted above a strongly basic resin (Dowex 1; X10; carbonate form; 1 cm × 12 cm long) as described by Gaull & Gaitonde (1967). After the passage of the sample the cation column was eluted with water (50 ml). The adsorbed amino acids from the Dowex 1 (X10; carbonate form) were then eluted with 0.1 M-acetic acid (60 ml). The eluate was evaporated to dryness and the residue dissolved in water (5 ml).

Colour development. To 1 ml of aqueous sample (containing 0.1–0.9 μmol of taurine) was added 1 ml of 0.06 M-phosphate (NaH_2PO_4–Na_2HPO_4) buffer, pH 6.4, and the contents were mixed. The tubes were placed in an ice bath for 10 min. This was followed by the addition of 1 ml of freshly prepared 50% (w/v) urea solution and the contents were thoroughly mixed. A 2% (w/v) ice-cold solution of *o*-phthalaldehyde (1 ml) was then added to all tubes, and after they had been mixed and left in ice for 5 min, 0.5 ml of acetic acid was added and the contents of the tubes were mixed by inversion. The tubes were left in ice for 30 min for colour development. The E_{560} was measured after the tubes had been left for 2–3 min at room temperature. We are grateful to Dr M. K. Gaitonde for giving us details of the method specific for the determination of taurine in brain tissue (Gaitonde & Short, 1971). The purple colour was stable for 2–3 min on reaching its maximum E_{560} value.

To check the number of amino acids we were eluting by this procedure the eluate from Dowex 1 (X10; carbonate form) was also analysed by an automatic amino acid analyser. We could detect only three ninhydrin-positive substances, of which only taurine was found to react with the *o*-phthalaldehyde reagent.

Uptake of taurine in vivo *in developing rat brain.* Immature (9 days old) and young adult (100–120 g) rats were injected intraperitoneally with 2 mg of taurine/g body wt. and the controls received an equal volume of 0.9% NaCl. After 1 h animals were injected with [U-^{14}C]-glycine (0.05 μCi/g body wt.). After a further 2 h the animals were killed by decapitation, their brains were then quickly removed, washed with 0.9% NaCl solution, dried with filter paper, weighed and frozen on solid CO_2. Taurine was extracted as described above. Radioactivity determinations were as described by Agrawal, Bone & Davison (1970).

Protein determination. An equal volume of 1 M-NaOH was added to samples from each subcellular fraction. The tubes were thoroughly mixed and incubated at 37°C for 18 h and protein was determined by the procedure of Lowry, Rosebrough, Farr & Randall (1951) as modified by Hess & Lewin (1965).

RESULTS

Changes in taurine concentration and cysteinesulphinate decarboxylase and glutamate decarboxylase activities in the developing rat brain. The high concentration of taurine in the newborn rat brain was found to decrease with increasing age, so that the concentration in the adult brain is about one-quarter that at birth (Table 1; Ramirez de Guglielmore & Gomez, 1966; Agrawal, Davis & Himwich, 1968).

Since taurine is formed via hypotaurine from cysteinesulphinate by decarboxylation (Peck & Awapara, 1967) it would be expected that the activity of this enzyme would parallel the changes in taurine concentration. However, there is very little cysteinesulphinate decarboxylase activity in the neonatal rat brain and the increase in this enzyme activity during maturation seems to be inversely related to taurine concentration (Table 1). There is also an increase during development of glutamate decarboxylase activity in the rat brain, but in this

Table 1. *Changes in taurine concentration and cysteinesulphinate decarboxylase and glutamate decarboxylase activities during development of rat brain*

Taurine was measured by the *o*-phthalaldehyde reaction and the decarboxylase manometrically as described in the Methods and Materials section. Results are given as mean ± S.D., with the number of observations in parentheses.

Age of rat (days)	Concn. of taurine (μmol/g)	Cysteinesulphinate decarboxylase activity (μl of CO_2/min per g)	Glutamate decarboxylase activity (μl of CO_2/min per g)
1	17.95 ± 0.66 (3)	0.43 ± 0.12 (3)	0.28 ± 0.04 (3)
6	14.40 ± 3.12 (3)	0.32 ± 0.12 (7)	0.71 ± 0.31 (8)
7	12.65 ± 2.16 (8)	0.67 ± 0.13 (6)	0.82 ± 0.16 (8)
17	7.09 ± 0.812 (4)	1.32 ± 0.27 (6)	2.53 ± 0.48 (5)
28	7.41 ± 0.50 (4)	1.50 ± 0.54 (6)	2.38 ± 0.53 (6)
Adult	4.65 ± 0.20 (8)	3.13 ± 0.62 (4)	4.85 ± 0.77 (8)

Table 2. *Subcellular distribution of taurine and decarboxylase activity in rat brain*

Taurine concentration and cysteinesulphinate decarboxylase and glutamate decarboxylase activities in 7-day-old and adult rat brain were assayed as described in the Methods and Materials section. Where no S.D. is given only two assays were performed; otherwise the number of determinations is given in parentheses. The fractions were prepared by the procedures of De Robertis *et al.* (1962, 1963). The decarboxylase activity in the 7-day-old rat brain was very low [0.75 ± 0.10 (3) and 0.73 ± 0.18 (3) μl of CO_2/h per g for cysteinesulphinate decarboxylase and glutamate decarboxylase respectively].

Fraction	Concn. of taurine (7-day-old rat) (μmol/g)	Concn. of taurine (adult rat) (μmol/g)	Cysteinesulphinate decarboxylase activity (adult rat) (μl of CO_2/h per g)
Whole-brain homogenate	12.72 ± 2.62 (3)	4.65 ± 0.20 (8)	3.13 ± 0.62 (4)
Nuclear pellet	0.58 ± 0.21 (3)	0.19 ± 0.005 (8)	0.19 ± 0.03 (4)
Crude mitochondria	2.06 ± 0.23 (3)	0.97 ± 0.24 (8)	1.69 ± 0.17 (4)
Supernatant	7.96 ± 0.32 (3)	3.43 ± 0.13 (8)	1.49 ± 0.24 (4)
Microsomal fraction	1.19 ± 0.38 (3)	0.19 ± 0.10 (8)	0.16 ± 0.01 (4)
Myelin	0.08 ± 0.001 (3)	0.27 ± 0.003 (3)	0.33 (2)
Membrane fragments	0.69 ± 0.04 (3)	—	—
'Light' nerve-ending particles	0.34 ± 0.18 (3)	0.23 ± 0.024 (3)	0.54 (2)
'Dense' nerve-ending particles	0.35 ± 0.05 (3)	0.25 ± 0.016 (3)	0.59 (2)
Purified mitochondria	0.30 ± 0.13 (3)	—	—
	After osmotic shock		
Membrane 'ghosts'		0.28 (2)	0.50 ± 0.04 (3)
Synaptic vesicles		—	—
Nerve ending + myelin supernatant		0.75 (2)	1.24 ± 0.36 (3)

case the concentration of the product, γ-aminobutyrate, shows only a small change during the same period (Agrawal *et al.* 1966).

The subcellular distribution of taurine and cysteinesulphinate decarboxylase is shown in Table 2 for adult and 7-day-old rat brain. In subcellular fractions from both adult and newborn brain taurine follows the distributions expected for a soluble amino acid (Ryall, 1964; Mangan & Whittaker, 1966). Thus about 70% of the taurine is found in the soluble fraction. Within the crude mitochondrial fraction taurine was equally distributed between myelin and each of the intact nerve-ending fractions in the adult brain. As was to be expected less uniform distribution was found in the 7-day-old brain. About 45% of the cysteinesulphinate decarboxylase activity in the adult was localized in the crude mitochondrial fraction, where its distribution in synaptosomes and myelin was similar to that of taurine. Enzyme activity in the fractions from 7-day-old brain was very low but it did appear that about 80% of both cysteinesulphinate decarboxylase and glutamate decarboxylase activity was membrane-bound. In the adult brain both taurine and cysteinesulphinate decarboxylase activity could be released from the crude mitochondrial fraction by osmotic shock (Table 2).

Anatomical distribution of taurine and decarboxylase activity. Considerable regional heterogeneity

Table 3. *Distribution of taurine, cysteinesulphinate decarboxylase and glutamate decarboxylase in different anatomical regions of adult rat brain*

Taurine and the decarboxylase activities were assayed as described in the Methods and Materials section. Results are given as mean ± S.D. with the number of observations in parentheses.

Region of brain	Concn. of taurine (μmol/g)	Cysteinesulphinate decarboxylase activity (μl of CO_2/min per g)	Glutamate decarboxylase activity (μl of CO_2/min per g)
Cortex	6.9 ±0.92 (3)	3.16±0.19 (6)	4.32±0.97 (6)
Cerebellum	5.79±0.86 (3)	2.05±0.37 (6)	2.60±0.60 (6)
Pons medulla	1.79±0.54 (3)	2.95±0.85 (6)	3.48±0.74 (6)
Midbrain	2.78±0.53 (3)	3.26±0.27 (6)	4.47±0.42 (6)

in the distribution of taurine was noted (Table 3) and there was no correlation in the concentration of the amino acid and its synthesizing enzyme. Roberts (1962) has observed a similar lack of correlation for the γ-aminobutyrate–glutamate decarboxylase system. There is an obvious similarity in the relative activity of cysteinesulphinate decarboxylase and glutamate decarboxylase in each region of the rat brain but no correlation between the taurine concentration and published values for γ-aminobutyrate (Roberts, 1962). Since Neame (1968) has suggested that taurine and glycine may to some extent be taken up by the same transport system (Lajtha, 1962; Battistin & Lajtha, 1969) we have undertaken a preliminary experiment and found no effect of raised plasma taurine concentration on entry of glycine and its incorporation into protein within the brain *in vivo*.

DISCUSSION

Although taurine is one of the most plentiful amino acids in the mammalian adult nervous system, its physiological role remains unknown (Gaitonde, 1970) as does the reason for its exceedingly high concentration at birth. We have shown that this concentration and its subsequent slow decrease during development of rat brain cannot be related to the activity of the synthesizing enzyme cysteinesulphinate decarboxylase. The decarboxylase shows a slow increase in activity during development from near negligible values in the newborn, to approximately half the adult activity at 28 days after birth. This pattern closely follows the developmental changes of glutamate decarboxylase activity. Two other possible explanations for the decrease in taurine concentration may be considered. Firstly, the original high concentration in the brain may simply reflect that in the maternal blood and the subsequent fall in concentration may be due to diffusion from the brain. On the other hand, an efficient and selective transport system for concentrating taurine may be operating in the young animal brain.

Our results on the subcellular distribution of taurine and cysteinesulphinate decarboxylase are similar to these found for γ-aminobutyrate and for glutamate decarboxylase (Ryall, 1964; Salganicoff & De Robertis, 1965; Fonnum, 1968); just under half of the enzyme being found in the nerve-ending particles. Thus in the adult at least, besides the ionophoretic evidence, another of the criteria for taurine being a neurotransmitter has been satisfied. Such a hypothesis is further reinforced by the obvious structural resemblance of taurine to glycine and γ-aminobutyrate, both of which have been implicated as neurotransmitters (Aprison, Davidoff & Werman, 1970; Krnjevic, 1970). The results on the anatomical distribution of taurine, cysteinesulphinate decarboxylase and glutamate decarboxylase lend support to this possibility. Unmyelinated nerve cells of the immature brain may be very sensitive to excitatory transmitters such as acetylcholine over almost all the cellular membrane surface (Harris & Dennis, 1970). At this stage of development taurine may well play a part in 'damping down' the electrical activity of these immature cells.

Other suggestions for the function of taurine also exist. Peck & Awapara (1967) have shown taurine to be slowly converted into isethionic acid in adult rat brain. Under the control of adrenaline and acetylcholine, isethionic acid is formed from taurine in dog heart, where it has a profound effect on K^+ retention (Welty & Read, 1964). If such a mechanism operates in the brain, especially in the young, any defect in taurine metabolism or transport, as may be found in mongolism (Thomas, Goodman, King & Wainer, 1965) may have serious consequences for the functioning of nerve cells. In analogy with experiments with phenylalanine (Agrawal *et al.* 1970) L. K. Kaczmarek (unpublished work) has tried to interfere with the normal balance of taurine transport by considerably raising plasma taurine concentrations and have shown that this has no effect on the transport or utilization in protein synthesis of a related amino acid, glycine. Alter-

natively it has been proposed that taurine may exert an effect on oxygen uptake (Sicuteri, Fanciullacci, Franchi, Giotti & Guidotti, 1970). Further work on the uptake and release mechanisms of taurine must be carried out before the true function of taurine in the central nervous system can be elucidated.

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