Journal of Neurochemistry, 1972, Vol. 19, pp. 431 to 435. Pergamon Press. Printed in Great Britain.

TAURINE DISTRIBUTION IN CAT BRAIN

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(*Received* 7 *June* 1971. *Accepted* 13 *August* 1971)

Abstract—The distribution of taurine was investigated in 16 areas of the brain, in cats transected either at collicular or at midpontine level. A comparison was also made between the content in the same areas of the cerebral cortex of the two groups of cats showing respectively either a synchronized or an activated electrocorticogram. Taurine was determined in picric acid extracts by means of column chromatography followed by thin layer chromatography of the eluates. The levels of taurine were fairly uniform in all areas investigated with the exception of the lateral geniculate bodies, the pituitary gland and the pineal gland where the levels were higher than in all other regions. The taurine content of the cortex of cats showing a synchronized EEG pattern was higher than in the cortex of cats showing an activated pattern. The results are discussed in the light of the limited information available on the possible role of taurine in the CNS.

THE EXTENSIVE review by JACOBSEN and SMITH (1968) on taurine describes the increasing interest in this sulphur-containing amino acid known for more than a century and identified in mammalian brain by STEIN and MOORE (1954) and by AWAPARA (1956). Taurine applied directly on single spinal neurons depresses the rate of neuronal firing with a potency similar to that of GABA (CURTIS and WATKINS, 1960). JASPER and KOYAMA (1969) demonstrated that taurine is released from the cerebral cortex of the cat and that the release is higher during arousal than during sleep. Both observations suggest a role of taurine in neuronal activity. TALLAN (1962) has reviewed papers reporting the levels of taurine in the brain of many animal species including man. In human brain OKUMURA, OTSUKI and KAMEYAMA (1960) investigated taurine distribution in seven regions and found the highest concentration in the frontal cortex and the lowest in the thalamus. PIHA, OJA and UUSITALO (1962) measured taurine content of four, SHAW and HEINE (1965) of six and SHANK and APRISON (1970) of seven regions of rat brain. Although the absolute values obtained by the different authors show some differences the distribution is similar in all reports with the highest concentration in the cerebral cortex and the lowest in the pons–medulla. On the other hand, in a study of taurine levels in five regions of cat brain, BATTISTIN, GRYNBAUM and LAJTHA (1969) found the highest concentration in the cerebellum and the lowest in the thalamus. In the present investigation taurine content was determined in 16 areas of cat brain and a comparison was also made between the contents of the same areas of the cerebral cortex of cats showing either activated or synchronized EEG patterns produced by different levels of midbrain transection.

MATERIALS AND METHODS

Twelve adult cats of both sexes were used. A tracheal cannula was inserted under halothane anaesthesia, the head was clamped in a stereotaxis apparatus and transections were made by means of a stereotactically oriented spatula either at midpontine pretrigeminal level, in order to obtain a persistently activated preparation, or at collicular level (cerveau isolé) in order to obtain a preparation

showing a synchronized EEG pattern. The anaesthesia was then discontinued and the cats resumed spontaneous respiration; blood pressure was recorded from the femoral artery by means of a pressure transducer and was always within the normal range. The temperature was controlled by means of a heating pad and was maintained at 37°C. The electrical activity of the cortex was recorded by means of screw electrodes implanted in the skull over the frontal and occipital areas of both hemispheres. The cats were killed by exsanguination 3 h after the transection. The skull was rapidly opened and the brain was carefully removed, leaving *in situ* the pituitary gland, and placed over crushed ice. Samples (10–200 mg) were dissected in three cats starting with the pineal gland. The hypophysis was then extracted. The entire procedure took about 10 min and the samples were immediately dropped into 1% (w/v) picric acid solution, weighed by difference and stored at −20°C until needed.

Tissue extraction. The samples were homogenized with 10 ml of 1% (w/v) picric acid solution/g of fresh tissue. The homogenates were boiled for 5 min and when cooled they were centrifuged at 3000 rev./min for 5 min. The supernatant fluid was decanted and passed through the chromatographic column.

Taurine determination. (a) Column chromatography. Taurine extraction was carried out using a modification of the method of HOPE (1957). The supernatant fluid was first passed through a column of cation exchange resin Dowex 2 × 8, 200–400 mesh in H^+ form, eluted with 1 per cent picric acid and the eluate was subsequently passed through a second column of 1 cm diameter and 2 cm height containing Dowex 2 × 8 200–400 mesh (OH^- form). Taurine is retained by this column while non-polar substances are discarded by washing with 50 ml of water. The taurine was then eluted with 30 ml of 1 N-HCl and the eluate was concentrated to about 1 ml, neutralized with NaOH and passed on third column of Dowex 50 × 8 200–400 mesh in the H^+ form and eluted with 10 ml of water. The eluate thus obtained was evaporated to dryness and the residue was taken up in a suitable volume, usually 0·2 ml, of water.

(b) Thin-layer chromatography of the eluates. Taurine was separated from other acidic amino acids and ninhidrin-positive substances on 0·25 mm silica gel G TLC plates. Suitable portions (5–40 μl) of tissue extract were applied to the plate. Of each unknown sample two samples, one twice the volume of the other, were applied to the adsorbant layer. Standards, of 1, 2 and 4 μl of a 0·25 per cent solution of taurine were applied to each chromatogram alternating with the unknown samples. The chromatograms were developed at 22°C for 3 h using butanol:acetic acid:water (60:20:20, by vol.) as the solvent system.

(c) Colorimetric determination. The dried chromatograms were sprayed for 45 s with a ninhidrin solution prepared according to HARRIS, MITTWOCH, ROBSON and WARREN (1955), containing 0·4 g ninhidrin, 95 ml acetone, 5 ml acetic acid and 5 ml water. After 30 min at room temperature the colour was developed by heating the chromatogram at 90°C for 30 min. The spots with the same RF as the standards of pure taurine were scraped off into tubes and extracted with 3 ml of methanol. After centrifugation at 2500 *g* the extinction of the supernatant fluid was measured at 510 nm. The extinction vs. standard taurine concentration was plotted and the amount of taurine present in the unknown sample was calculated from these curves. The results from 25 different calibration curves were combined and statistically analysed. The equation of the regression line was as follows: $\bar{y} = 0{\cdot}005 + 0{\cdot}019x$. The fiducial limits with a probability of 95 per cent indicate an error of ±4 per cent. According to AWAPARA (1956) and HOPE (1957) taurine is quantitatively recovered from the columns used in our method. Doses of 50, 150 and 300 μg of taurine added to brain samples and analysed by the above procedure gave a percentage recovery of 90 ± 0·7, 89 ± 0·5 and 88 ± 0·9 respectively (n = 3).

RESULTS

The results reported in Table 1 show that the levels of taurine were rather similar in all areas investigated with the exception of the lateral geniculate bodies, the pituitary gland and the pineal gland in which the levels were higher than in all other regions. The significance of the difference between these values and all other values was determined by analysis of variance and $P < 0{\cdot}01$ was obtained. The corpora quadrigemina, the medial geniculate bodies and the spinal cord showed the lowest taurine contents. In the groups of values reported in Table 1, no distinction was made as to whether they were obtained from cats showing either an activated or a synchronized EEG. In Table 2 a comparison is made between the contents of taurine from samples excised from the cerebral cortex of two groups of cats transected at different levels. The cats of one group were transected at midpontine pretrigeminal level and their

TABLE 1.—TAURINE DISTRIBUTION IN VARIOUS REGIONS OF THE CAT BRAIN

Region		Taurine content (μmol/g ± S.E.M.)	Region		Taurine content (μmol/g ± S.E.M.)
			Lateral geniculate b	(7)	4·34 ± 0·65
Cerebral cortex					
Frontal	(5)	2·05 ± 0·68	Medial geniculate b	(1)	1·66
Parietal	(5)	2·28 ± 0·52	Corpora quadrigemina	(4)	1·74 ± 0·28
Occipital	(5)	2·09 ± 0·23	Pons	(2)	2·14 ± 0·48
Temporal	(5)	2·09 ± 0·23	Medulla oblongata	(3)	1·73 ± 0·67
Cerebellum	(4)	2·68 ± 0·37	Spinal cord	(3)	1·76 ± 0·62
Caudate	(6)	2·91 ± 0·66	Pituitary gland	(8)	5·20 ± 0·91
Thalamus	(7)	2·27 ± 0·30	Pineal gland	(3)	8·14 ± 0·45
Hypothalamus	(6)	1·81 ± 0·10			

Number of determinations in brackets. The significance was determined by the analysis of variance. The difference between content of the three underlined values and that of all other values is statistically significant.

EEG pattern was characterized by low amplitude high frequency waves typical of wakefulness. Ocular movements following an object moving vertically were frequent and were further evidence of the alert behaviour of the cat. The cats of the other group were transected at collicular level and their EEG pattern was characterized by slow waves and spindle burst. The pupils were miotic. The taurine content of the cortex of the latter group was higher than in the activated group, however the difference was statistically significant only in the temporal lobe.

TABLE 2.—TAURINE CONTENT IN THE CEREBRAL CORTEX OF CATS WITH TRANSECTIONS AT DIFFERENT MIDBRAIN LEVELS

Level of the transection	Midpontine pretrigeminal	Collicular
EEG Pattern	Activated	Synchronized
Cortical regions	Taurine content (μmol/g ± S.E.M.)	Taurine content (μmol/g ± S.E.M.)
Frontal	2·00 ± 0·14 (4)	2·13 ± 0·21 (6) n.s.
Parietal	2·19 ± 0·37 (6)	2·36 ± 0·27 (8) n.s.
Occipital	1·70 ± 0·19 (5)	2·14 ± 0·16 (6) n.s.
Temporal	1·97 ± 0·14 (4)	2·84 ± 0·15 (6) $P < 0·01$

Number of determinations in brackets.

DISCUSSION

TEWS, CARTER, ROA and STONE (1963) demonstrated that the taurine content of the dog brain is not affected by anoxia and does not change within 20–23 min after death. Therefore the dissection of the samples in our experiments should not have altered the actual level of taurine at death. The levels of taurine found in this investigation are

in good agreement with those reported for the cat by TALLAN, MOORE and STEIN (1954), by PORCELLATI (1963) and by BATTISTIN *et al.* (1969). The last authors found the lowest content (1·06 μmol/g) in the thalamus and the highest (3·12 μmol/g) in the cerebellum. Similar contents of taurine were found in the rat brain with the lowest level in the pons–medulla, according to PIHA *et al.* (1962), to SHAW and HEINE (1965) and to SHANK and APRISON (1970). In dog brain (TEWS *et al.*, 1963) and in the human brain (OKUMURA *et al.*, 1960) the concentration of taurine is in the range of 1–2 μmol/g. In three regions of the cat brain which had not been previously investigated, namely the lateral geniculate bodies, the pineal gland and the pituitary gland, we found a taurine content which was about two, two and a half and four times higher than in the cortex. VELLAN, GJESSING and STALSBERG (1970) observed that taurine is the amino acid present in highest concentrations in human pineal and pituitary glands. The level of 3 μmol/g reported by these authors is approx. twice as high as the content of taurine in the frontal lobe in human brain according to OKUMURA *et al.* (1960).

The high concentration of taurine in the lateral geniculate bodies and the pituitary and pineal glands is difficult to interpret. A possible role of taurine in the optic pathways is also suggested by the finding that a large amount of taurine occurs in the retina of many species (KUBICEK and DALENEK, 1958). In cats transected at collicular level the ACh content is higher (PEPEU and MANTEGAZZINI, 1964) and GABA content is lower (PEPEU, BARTOLINI and BARTOLINI, 1970) than in those transected at midpontine pretrigeminal level. In this investigation we observed that in some cortical areas of cats transected at collicular level and showing a synchronized EEG, the taurine content is higher than in cats transected at midpontine level. The differences are rather small in the frontal, parietal and occipital regions, reaching statistical significance only in the temporal cortex. They may be considered in keeping with the relatively small differences in taurine output from the sensory cortex between the right and left hemispheres of cats in which the left mesencephalic reticular formation was electrocoagulated and the right one was stimulated, observed by JASPER and KOYAMA (1969). On the other hand, the same authors observed a striking 10-fold increase in the liberation of taurine during arousal in neuraxially intact animals.

According to BATTISTIN *et al.* (1969) a comparison of the relative distribution of taurine *in vivo* and its uptake *in vitro* in some areas of the cat brain showed no parallelism. No other information on regional differences in taurine metabolism in the brain is available at the moment making it difficult to draw any definite conclusion on the meaning of our findings.

Acknowledgements—This work was supported partly by a grant of the Consiglio Nazionale delle Ricerche, Roma and partly by a gift from the Laboratorio Farmochimico Falorni, Florence, Italy.

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