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Observations on the relationship between the extracellular changes of taurine and glutamate during cortical spreading depression, during ischemia, and within the area surrounding a thrombotic infarct

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Summary. Taurine and glutamate were monitored by microdialysis technique during various cerebral insults: a. Application of K^+ triggered a cortical spreading depression (CSD). Taurine and glutamate increased concomitantly but recovery of glutamate was faster than that of taurine. b. Application of NMDA induced also CSD but only taurine increased. c. Induction of an infarct triggered repetitive CSDs. Taurine increased rapidly whereas glutamate rose slowly starting with some delay. d. After induction of ischemia, taurine and glutamate increased after onset of depolarisation. The increase of glutamate occurred late after a small, transient increase in parallel with the depolarisation. These data suggest a close functional relationship between the changes of both amino acids. Therefore, they should be monitored together especially in clinical settings: during excitation, only taurine will increase; during overexcitation, taurine will also increase but to a higher maximum followed by a moderate rise of glutamate; after energy failure, taurine will accumulate to its highest level followed by a continuous rise of glutamate.

Keywords: Amino acids – Spreading depression – Infarct – Ischemia – Microdialysis – DC potential

Introduction

In brain physiology, glutamate plays a dual role: On the one hand, it is a metabolite involved in many pathways (Kvamme, 1998); on the other hand, it is the most wide spread excitatory neurotransmitter in mammalian brain exhibiting toxic properties when increased beyond a certain threshold (Olney, 1994). On the contrary, the sulfur-containing amino acid taurine represents a metabolic endpoint (Brand et al., 1998) and shows ambivalent activities either as an inhibitory transmitter and/or as an osmoregulator (Bradford et al., 1978; Oja and Saransaari, 1996). Both amino acids share a high intracellular concentration. Glutamate is compartmentalised additionally into vesicles but taurine

is not (isolated synaptic vesicles do not take up taurine (Fykse and Fonnum, 1996 [ID: 2171]). Both amino acids also share a high concentration within the extracellular space, with the glutamate levels being somewhat lower than those of taurine as measured by microdialysis. However, the concentration of glutamate is very high compared to other known transmitter systems.

Although the release mechanisms of glutamate in respect to its role as neurotransmitter are rather clear (Fillenz, 1995), the control of the particularly high extracellular concentration is not understood so far. Glutamate can increase under various conditions (Timmerman and Westerink, 1995). A decrease, however, has rarely been observed. Several transporters are involved in the uptake- and release processes which have different properties depending on their location on the soma or the synapse (Billups et al., 1998; Danbolt et al., 1998).

Elevation of extracellular glutamate triggers influx of calcium and sodium into the cells (Olney, 1994) and thus can cause cell swelling (Choi et al., 1989) and cortical spreading depressions [CSD; (Leao, 1944; Bures et al., 1974)]. During ischemic conditions, glutamate increases within the extracellular space to an extent causing irreversible cell damage (Benveniste et al., 1984). Therefore, glutamate has been suggested to be monitored in patients during cerebral insults as stroke and trauma (Stjernstrom et al., 1993). However, due to its various sources the relevance of that parameter has been called into question (Timmerman and Westerink, 1997).

By contrast to glutamate, taurine is a less known amino acid and its role in the brain is less clear. It is metabolised from cysteine rather slowly (Brand et al., 1998) and has to be taken up by food to a certain extent varying between different species (Gaull et al., 1985). Lack of taurine during e.g. early development leads to blindness and neurological disorders (Rassin et al., 1978). Functionally, taurine has been attributed as an inhibitory transmitter (Schousboe et al., 1991; Galarreta et al., 1996). It has also been shown to play an important role during osmoregulation (Schousboe and Pasantes-Morales, 1992).

Thus, although less known and largely neglected in pathophysiological studies, taurine may serve various requirements during a brain cells life (van Gelder, 1983; Huxtable et al., 1994). Especially its possible role as a counterpart of the glutamatergic activities has not been addressed so far. Since we observed indeed a close relationship between the extracellular changes of glutamate and taurine in various studies on brain pathophysiology, the present paper has at its aim to summarise those observations. Comparing those observations, the discussion will focus on a search for a common denominator mediating or modulating the changes of both amino acids and to suggest the inclusion of both amino acids during clinical monitoring of cerebral insults.

Material and methods

All experiments were conducted according to the recommendations of the Declarations of Helsinki and Tokyo and to Guidelines for the Use of Experimental Animals of the

European Community and the actual versions of the German Tierschutzgesetz. The experimental protocol was approved by the local authorities and the local ethical committee.

Surgical procedure: Male Wistar rats (220–280 g) were anaesthetised with urethane (1.80 g/kg) and fixed in a stereotaxic frame. A burr hole of 3 mm diameter was drilled through the skull. A microdialysis (MD) probe (2 mm length, 0.5 mm outer diameter, Carnegie Medicine, Stockholm Sweden) was implanted in the parietal cortex behind the bregma. As perfusate artificial cerebrospinal fluid (CSF; composition: NaCl 125 mM; KCl 3 mM; CaCl2 1.1 mM; MgCl2 0.8 M; Na2HPO4 0.5 mM; NaHCO3 25 mM; D-glucose 6 mM) was used at a flow rate of 2 μl/min. Within a distance of 50–150 μm a microelectrode at a depth of 1 mm for measurement of direct current (DC) was implanted. Body temperature was maintained at 37°C with a water jacket. After an equilibration period of 90 min after insertion of the microdialysis probe, the various experimental maneuvers were performed (see below).

Physiological variables: The following parameters were recorded and monitored during each experiment: mean arterial blood pressure (using a Statham, Oxnard, CA, USA); ECG (recorded with two electrodes fixed subcutaneously to the left fore paw and the right hind paw) and heart rate (calculated from the ECG); blood gases at the beginning and twice in the course of the experiment (O_2, CO_2, pH; Eschweiler System 2000 (Eschweiler, Kiel, Germany)). In addition, the EEG and/or the ECoG were recorded in order to monitor depth of anaesthesia and confirm the spreading depressions as recorded by the microelectrode.

Experimental protocols

Local application of K^+ or NMDA: CSF containing 128 mM K^+ (replacing the NaCl by KCl) was perfused locally for 1 to 3 min. With interstimulus intervals of 60 min, the high K^+-CSF could be repetitively applied. Alternatively, CSF containing 10 mM NMDA was applied for 30 s. It also could be applied repetitively at interstimulus intervals of 60 min without any obvious signs of damage. Using flow rates of 2 μl/min and collecting 2 μl, a time resolution of 1 min was achieved.

Complete ischemia: At the end of each experiment, cardiac arrest was induced by intravenous injection of 0.3 ml saturated $MgCl_2$. With the same flow rates and collection volumes (e.g. 2 μl), postischemic periods up to 30 min were monitored.

Thrombotic infarction: After drilling a burr hole above the parietal cortex, an optic fibre (OD 5 mm; Dolan-Jenner) was positioned anterior to it. The microdialysis (MD) probe was implanted into the supposed peri-infarct area at an angle of about 30° in the sagittal plane. The DC electrode was positioned at a distance of about 50 to 100 μm from the probe and at a depth of 1 mm. After the usual equilibration period, Rose Bengal was given i.v. (70 mg/kg over 2 min) and the light switched on for 5 min (30 to 35 kLux). Fractions of 20 μl were collected over an experimental period of 6 h.

Analytical procedure: Amino acids were determined by HPLC with fluorescence detection after automated precolumn derivatisation with o-phthaldialdehyde (HPLC column: 125 × 3 mm Multosphere 100-18-5/FBS, particle size 5μm; mobile phase A: 12% B in 0.1 mol/l Na-acetate buffer, pH 5.4; mobile phase B: acetonitrile 30%, methanol 30% water 40%; linear step gradient (min/B %: 0/10; 17/25; 19/35; 23/50; 24/100; 31/100; 32/10; flow rate 0.6 ml/min). In some instances, not only glutamate and taurine but also aspartate were monitored. However, aspartate seemed to change in parallel to glutamate and its determinations, therefore, were omitted later on.

Data presentation and statistics: The amino acid levels are given as dialysate concentrations; no further extrapolations on extracellular concentrations were performed. Data are always given in means +/− standard deviations. Wilcoxon-Mann-Whitney-U-test was used for statistical analysis in results sections 2.

Results

Physiologic variables: Mean heart rate varied between 380 and 470 bpm. Mean arterial blood pressure ranged from 80 to 125 mmHg. Mean blood pH varied from 7.35 to 7.4. Mean pCO_2 ranged from 38 to 55 mmHg. Mean oxygen saturation varied between 135 and 177 mmHg.

1 Application of high potassium

Local application of high potassium via the microdialysis probe into the cerebral cortex for 3 min induced a single spreading depression occurring with a latency of 81.5 ± 20.2 s, with an amplitude of about 18.3 ± 3.6 mV and with a duration of 28.0 ± 10.1 s (Fig. 1). Recovery occurred spontaneously sometimes even in the presence of the high K^+. Insertion of a second electrode in some experiments allowed to determine the propagation of the CSD towards remote tissue areas with a speed of 3.5 ± 1.1 mm/min. Aspartate, glutamate and taurine started to increase concomitantly with the diffusion of K^+ into the cortex as detected by the DC deflection. An example is given in Fig. 1. Taurine increased from a basal level of 1.53 ± 0.41 μmol/l to a maximum of 6.09 ± 1.52 μmol/l. Glutamate increased from 0.75 ± 0.78 μmol/l towards 5.24 ± 2.24 μmol/l. In most instances, aspartate and glutamate reached their maximum prior to or concomitantly with the maximum of the DC deflection. Taurine usually peaked 1 to 2 fractions later. The peak area of taurine was 43.5 ± 13.7 μmol. Aspartate and glutamate returned to basal levels within 6.3 ± 1.5 min and 6.4 ± 1.5 min respectively. Taurine reached its basal level after

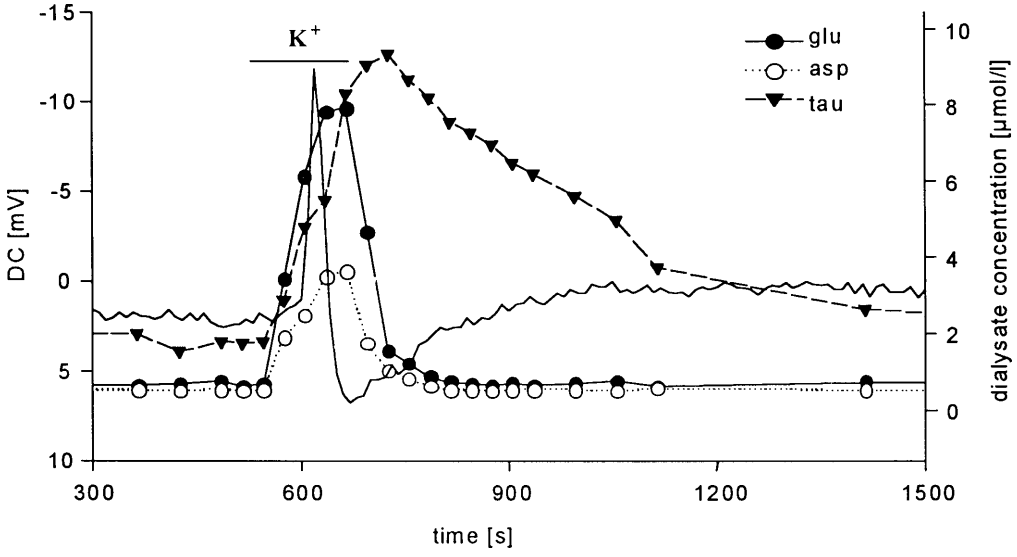

Fig. 1. A single, representative experiment illustrating the K^+-induced cortical spreading depression (line only) and the accompanying sequential changes of aspartate (open circles), glutamate (filled circles) and taurine (filled triangles) in the dialysate fractions. Means ± standard deviations are given in the text and Table 1

22.3 ± 4.8 min. Repetitive applications of K^+ did not affect the shapes of the subsequent CSD's but the amount of amino acids released tended to decrease.

2 Application of NMDA

Local application of NMDA via the microdialysis probe for 30 s always induced a single spreading depression with a latency of 32.3 ± 14.7 s. The sequence of events in an individual experiment is depicted in Fig. 2. The shape of the CSD as well its propagation velocity was not different from that observed after potassium application. The main difference was related to aspartate and glutamate: both amino acids did not change at all during the induction of CSD's by NMDA. Since aspartate has not been measured regularly, only the glutamate data are given in Table 1 and Fig. 2. On the contrary,

Table 1. Dialysate concentrations of taurine and glutamate after K^+ or NMDA-induced CSD, during complete, irreversible ischemia and within the peri-infarct zone (in means ± standard deviations; n indicates the number of individual experiments)

	Taurine			Glutamate		
	basal level	maximum	n	basal level	maxium	n
K^+-ind. CSD	1.53 ± 0.41	6.09 ± 1.52	22	0.75 ± 0.78	5.24 ± 2.24	21
NMDA-ind. CSD	1.36 ± 0.36	5.79 ± 1.82	19	0.69 ± 0.80	0.81 ± 0.72	16
ischemia	1.29 ± 0.58	28.14 ± 8.64	14	0.56 ± 0.32	8.73 ± 8.08	13
peri-infarct zone	2.77 ± 0.44	16.04 ± 11.08	8	0.84 ± 0.41	4.58 ± 1.77	8

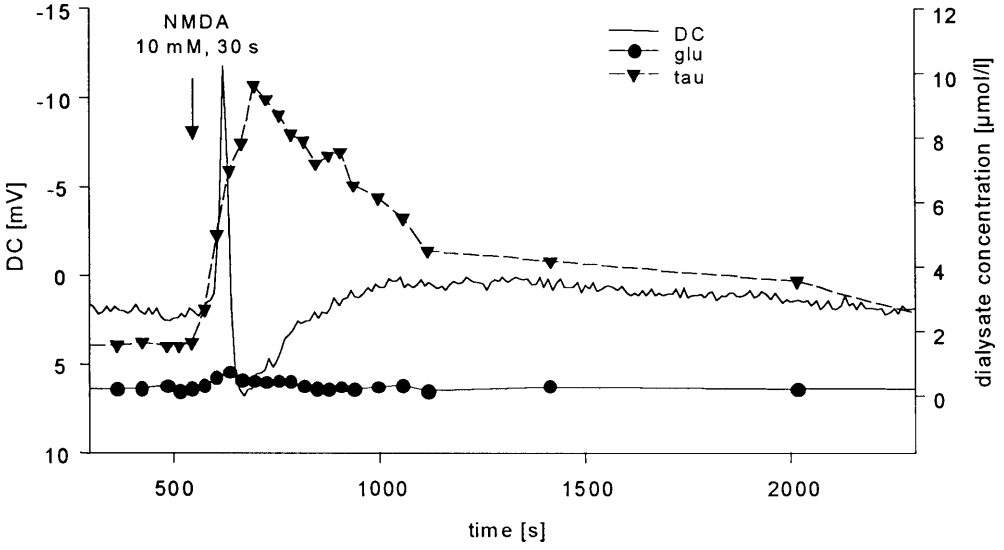

Fig. 2. A single, representative experiment illustrating the NMDA-induced cortical spreading depression (line only) and the accompanying sequential changes of glutamate (filled circles) and taurine (filled triangles) in the dialysate fractions. Means ± standard deviations are given in the text and Table 1

taurine increased and decreased with about the same kinetics as under K^+ (Fig. 2) starting from a basal level of 1.36 ± 0.36 μmol/l and increasing towards 5.79 ± 1.82 μmol/l. The peak area was somewhat smaller (35.4 ± 11.5 μmol) but was borderline regarding statistical significance. Repetitive NMDA applications resulted in CSD's not different from the 1st CSD although the amount of taurine release tended to decrease.

3 Induction of ischemia

After induction of ischemia, the DC signal showed a negative deflection of 19.6 ± 2.3 mV with a latency of 101.1 ± 21.12 s (Fig. 3a). Prior to this DC shift, glutamate, aspartate and taurine did not change much from a basal level of 0.56 ± 0.32, 0.54 ± 0.35 and 1.29 ± 0.58 μmol/l respectively (Table 1). However, together with the negative DC shift, aspartate and glutamate increased only transiently (13-fold for asp in n = 6 out of 9 experiments and 4-fold for glutamate in n = 4 out of 9 experiments) and recovered to the pre-ischemic level within about 2 min. Again, representative experiments are shown in Fig. 3a and 3b. With a further delay of 2 to 3 min, both amino acids started to increase again but this time rose very slowly. Aspartate increased again about 13-fold whereas glutamate increased 15-fold during the next 30 min (Table 1). On the contrary, taurine started to rise about 1 min after the negative DC shift occurred and thus earlier than aspartate and glutamate and reached a final level about 22-fold above base line (Table 1). A drug-induced delay of the negative DC shift was also accompanied by a delay of the peaks of glutamate and taurine (Scheller et al., 1990). For comparison of the changes of taurine and glutamate during K^+-induced CSD and ischemia, the dialysate concentrations of both amino acids of an individual experiment are shown in Fig. 3b. The fig. also illustrates the asymptotic time course of taurine approaching a final, maximal level during the experimental period during which glutamate still continued to increase.

4 Photothrombotic infarct

Illumination of the circulating dye Rose Bengal resulted in the occurrence of CSDs with a latency of 123.13 ± 12.22 s (n = 8). In Fig. 4 a representative experiment is shown. They lasted for up to 3 hours and amounted to 13.75 ± 12.61 CSD's (range: 3 to 37).

Extracellular glutamate started to rise between 20 to 30 min after onset of illumination (Fig. 4) and rose from 0.84 ± 0.41 μmol/l before light onset to 4.58 ± 1.77 μmol/l at 5 hours after light onset (Table 1). Extracellular taurine started to rise immediately at the onset of illumination, at about the time of the first spreading depression, and reached its maximum level within 20 to 30 min after light onset (Fig. 4). The initial rise of taurine was typically faster than that of glutamate and started from a level of 2.77 ± 0.74 μmol/l prior to illumination to a maximum of 16.04 ± 11.08 μmol/l (Table 1) and decreased

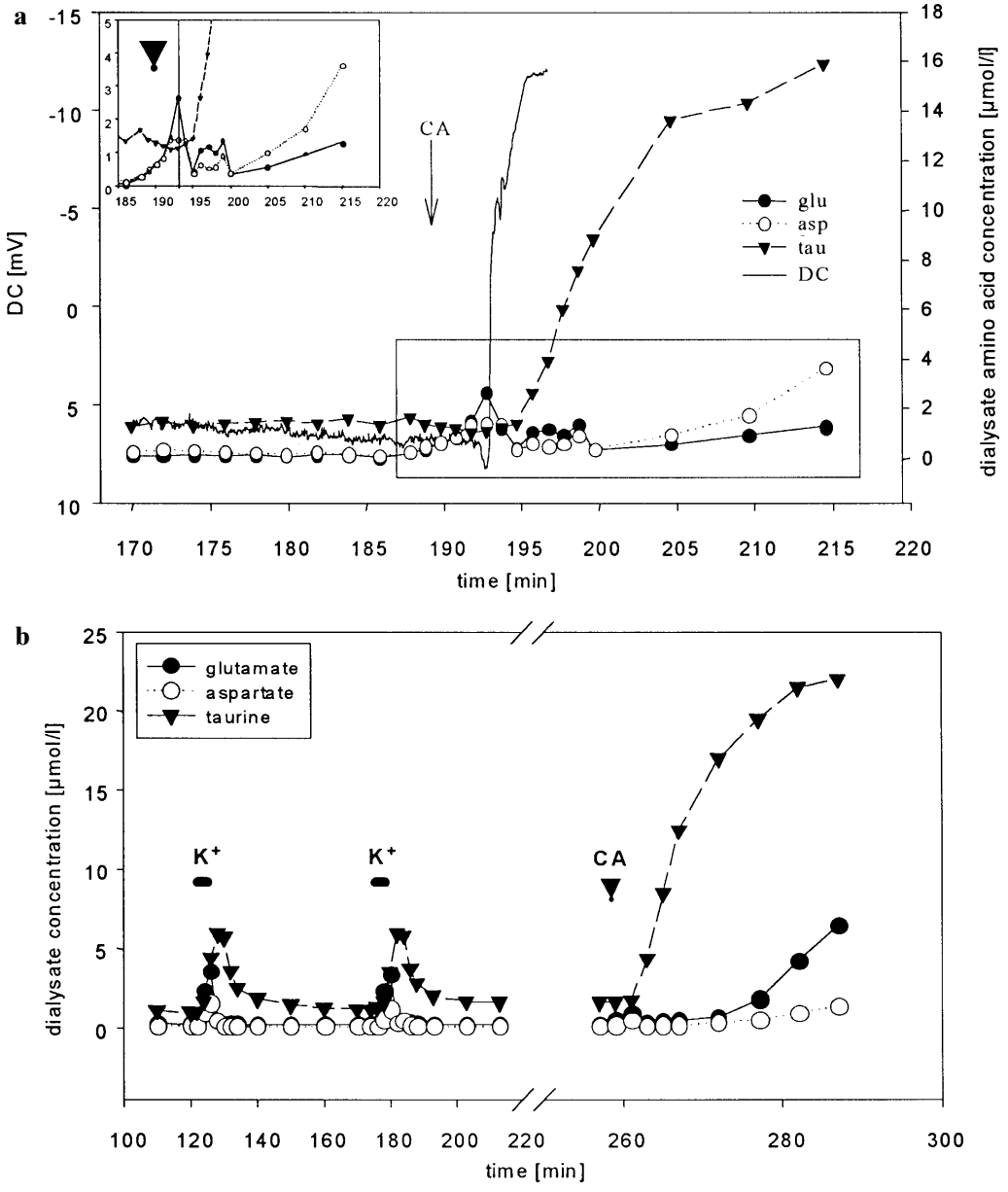

Fig. 3. Extracellular changes of the amino acids. **a** Changes in relation to the DC signal: A single, representative experiment illustrating the changes of aspartate (open circles), glutamate (filled circles) and taurine (filled triangles) after induction of a complete, irreversible ischemia (arrow, *CA*). Three minutes later, the DC potential exhibits the anoxic depolarisation (negative shift of 21 mV). On the inset, the period around the anoxic depolarisation of the DC potential is redrawn at an enlarged scaling of the y-axis. The arrow indicates onset of ischemia, the dotted line refers to the anoxic depolarisation. **b** Changes of the amino acids during CSD and ischemia: For comparison a representative experiment is illustrated showing the increases of glutamate, aspartate and taurine (symbols as in Fig. 3a). For quantitative data see Table 1

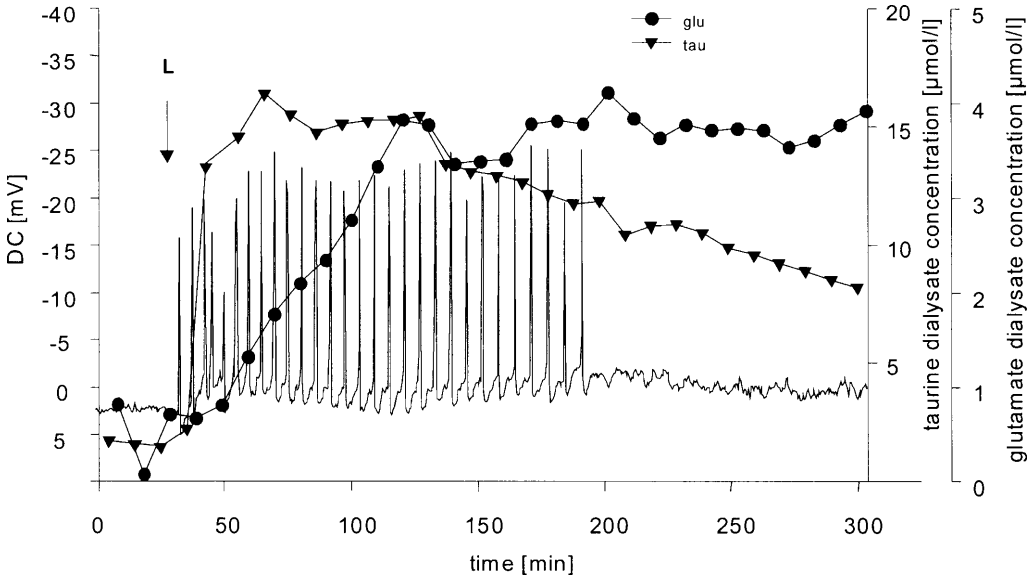

Fig. 4. A single, representative experiment illustrating the time course of events after induction of a thrombotic infarct: After onset of illumination (arrow, *L*), spreading depressions (line only) were monitored first followed by a rapid increase of taurine (filled triangles). Glutamate (filled circles) started to increase slowly and with some delay and reached a plateau whereas taurine tended to decrease. The decrease of taurine was in average not as obviously as shown here. Means ± standard deviations are given in the text and Table 1

slowly afterwards to a final level of 10.75 ± 3.77 μmol/l at the end of the experiment.

Discussion

The paper summarises the extracellular dynamics of taurine and glutamate occurring during cortical spreading depression (CSD), during complete ischemia and in the zone surrounding a thrombotic infarct, the so-called penumbra or peri-infarct zone. The measurements were performed by means of the microdialysis technique with a high time resolution of 1 min in relation with the recording of the DC potential. This allows not only to detect the changes in more details than described so far but also to follow the sequence of events. The results illustrate that taurine and glutamate change in a close correlation during a pathological insult although they are seemingly not directly linked.

The most consistent finding was that taurine increased under all circumstances investigated – during CSD, during ischemia and in the peri-infarct zone. Its rise occurred rapidly after onset of the insult and with obviously similar kinetics: during CSD and in the peri-infarct zone, taurine increased immediately after the induction of the insult (Figs. 1, 2 and 4). The maxima reached, however, were different (Table 1). Under both conditions the cells

are supposed to be stressed but still reactive. The maxima probably indicate new steady states of different degrees of release and re-uptake activity which might be called an "excitatory" (as during CSD, Figs. 1 and 2) and an "overexcitatory equilibrium" (as in the peri-infarct zone, Fig. 4). During complete and irreversible ischemia, taurine started to increase with some delay after the onset of the insult but more or less concomitantly with the anoxic depolarisation (Fig. 3a). It rose continuously and asymptotically towards a maximum much higher than under the conditions mentioned before (Fig. 3b). After 30 min of complete ischemia, when all cells are irreversibly damaged (Hossmann, 1994; Hossmann et al., 1983), the maximum achieved probably indicates a complete equilibration of taurine between the intra- and the extracellular space (and might be called "ischemic equilibrium"; Fig. 3b). From that point of view, the dialysate concentrations of taurine can give a hint for the functional state of the tissue: excitation (as during CSD), overexcitation (as in the peri-infarct zone), and energy depletion (as in ischemia); in the latter case, the maximum levels in the dialysate will be highest whereas for the former different degrees will be found. Thus, taurine could be a valuable parameter to monitor clinically the severity of an insult by microdialysis.

In contrast to taurine, glutamate exhibited a more complex pattern of changes: Glutamate increased rapidly during K^+-application (Fig. 1), but increased rather slowly and with some delay in the peri-infarct zone or during complete ischemia (Figs. 3a,b and 4). In earlier reports, glutamate had been shown to increase immediately with the onset of ischemia [(Benveniste et al., 1984) ID: 2061]. In the present experiments, we observed an increase starting with some delay after cell depolarisation occurred and subsequent to a small, but transient elevation of glutamate in the early ischemic period (Fig. 3a, insert), which has not been described so far and which will be discussed later (see below).

In contrast to the conditions mentioned so far, glutamate did not increase after NMDA application although a CSD occurred (Fig. 2) as after K^+-application. This suggests that the changes of glutamate are not related to the CSD but to the stimulus used: NMDA is supposed to predominantly activate its post-synaptic receptor and thus to induce a CSD whereas K^+ can have a variety of effects: due to the diffusion across the microdialysis membrane, the K^+ concentration will be low in the initial phase of application but it will steadily increase. Initially, the depolarisation of the cells will be only moderate but sufficient to trigger a CSD (which is supposed to occur via Ca^{++}-mediated glutamate release because the CSD can be blocked by NMDA antagonists (Marrannes et al., 1988; Sheardown, 1993)). Later on, K^+ will rise further and depolarise neurones and glial cells to about the same extent. As a consequence, the synaptic release will be enhanced further but at the same time the re-uptake mechanisms will be blocked or even turned backward (Fillenz, 1995; Billups et al., 1998). Thus, K^+ not only triggers the release of glutamate, but also contributes to its detectability by limiting the re-uptake. From that point of view, it will be difficult to separate the rate of synaptic glutamate release after K^+-application from the K^+-mediated non-synaptic

release. This difficulty raised questions on whether the determination of glutamate by microdialysis can give valuable information on its function as a transmitter (Timmerman and Westerink, 1997). Under more physiological conditions, re-uptake blockers might be taken into consideration instead of K^+ in order to differentiate between neuronal and glial glutamate release because it will be more specific than the elevation of K^+. In respect to pathophysiological investigations, however, its increase should not be attributed to synaptic release only. It should be kept in mind that at the same time the extracellular K^+ concentration will be elevated and exert various effects on release and re-uptake.

In contrast to broader effects of K^+, the more specific action of NMDA at its receptor induces a short lasting cell depolarisation only. Since the accompanying glutamate transients supposedly are very fast and the re-uptake systems still continue to work undisturbed (Danbolt et al., 1998), the depolarisation mediated glutamate release is not detectable under those circumstances. Glutamate may only become observable by means of adding a re-uptake blocker to the perfusate. Those experiments, however, still need to be done.

In contrast to glutamate, the CSD-mediated increase of taurine is independent from the trigger used and exhibits comparable kinetics (Figs. 1 and 2) and comparable elevations (Table 1). This suggests that it specifically indicates the CSD-mediated cell swelling (Schousboe et al., 1990) and thus might be a measure for the compensatory activity after pronounced excitation.

The rapid increase of taurine within the peri-infarct zone, therefore, might be attributed to the overexcitation mediated by the series of CSDs. This is reflected by the higher levels reached in that zone (Table 1) compared to single CSDs. Having passed the maximum, taurine slowly decreases. Glutamate seems to increase after taurine has passed its maximum suggesting that the release of taurine could have contributed to the glutamate re-uptake. This could have occurred in spite of the fact that the ion gradients are transiently abolished during the series of CSDs (Hansen, 1985). Although the taurine gradients may flatten afterwards, it may equilibrate over a time period as the duration of the present experiments. The remaining gradient may still be sufficient to drive anion exchange systems which indirectly may carry on the glutamate re-uptake.

However, other factors contributing to the rise of glutamate have to be also considered, e.g. leakage or diffusion of K^+, glutamate or other (excito-) toxins out of the infarct core into the peri-infarct zone (Phillis et al., 1994; Szatkowski and Attwell, 1994). Due to a persistent overexcitation and the corresponding metabolic stress, the energy stores may finally become depleted. The energy depletion will contribute to further disturbances of the homeostasis and thus a futile cycle will be initiated. At its end, the cells in the peri-infarct zone will die (van Reempts et al., 1987; Hossmann et al., 1983; van Reempts and Borgers, 1994; Hossmann, 1994; Szatkowski and Attwell, 1994) and glutamate and taurine are expected to increase further as e.g. occurring during complete ischemia. Under that condition, taurine rises to a very high level, the ischemic equilibrium. Under that condition, taurine can not contrib-

ute any more to anion-exchange-mediated glutamate uptake and thus glutamate may increase even further – up to 100 times its basal level (Benveniste et al., 1984). To that end, glutamate from various sources may contribute as e.g. being cleaved from proteins. Under our experimental conditions, those high glutamate have not been reached (Table 1). This phenomenon also deserves further investigations.

Before its ischemic rise, glutamate (and in parallel aspartate) has exhibited a short lasting transient increase occurring concomitantly with the anoxic DC shift which indicates a more or less synchronous depolarisation of the cells. This is a very interesting observation and might in accordance with the above mentioned events be explained as follows: After induction of ischemia, ATP starts to decline (Siesjö, 1978); during that initial period, marked transmitter and ion fluxes occur which, however, still are compensated for – at least for a short period of time (Hansen, 1985); when ATP has dropped to about 30% of its normal level, the ischemic depolarisation takes place accompanied by a steep rise of extracellular K^+, a drop of extracellular Na^+ and Cl^- and a massive influx of Ca^{++} into the cells (Hansen, 1985; Katayama et al., 1991). Subsequently, a Ca^{++}-mediated glutamate release from the transmitter pool takes place (Katayama et al., 1991). The rise of K^+ plus the beginning energy deficit in the initial phase of the ischemia restricted the re-uptake of glutamate but not completely and thus allowed synaptically released glutamate to be detected by microdialysis. Now taurine becomes released, too. The ongoing re-uptake still seems to be as efficient as to remove the released glutamate completely (at least during a short time period). However, the ion gradients are completely abolished at that time (Hansen, 1985) and thus taurine might come into play exchanging with other anions (Cl^-/HCO_3^-, amino acids, keto-acids) (Szatkowski and Attwell, 1994; del Rio et al., 1996) and thereby contributing indirectly to the removal of glutamate from the extracellular space. Under the ischemic conditions, however, the release of glutamate will occur as rapid that the flattening gradient of taurine may not be sufficient to keep the system running for a prolonged period of time. Glutamate, therefore, starts to increase again. As a consequence, once taurine has reached its ischemic equilibrium the anion-exchange systems do not work any more and this will mark the point of no return. This possible role of taurine has not been addressed so far but may be worth studying further. At least this observation may be helpful illustrating the necessity to determine taurine in addition to glutamate in clinical monitoring.

In conclusion: The determination of taurine and glutamate by microdialysis technique with a high time resolution gave a more detailed insight into the sequence of the events occurring during periods of overexcitation and energy depletion. During overexcitation, taurine is released and its extracellular concentration reaches a new equilibrium related to the degree of excitation; Glutamate, however, remains low or increases only slowly. During energy depletion, extracellular taurine and glutamate will remain low as long as the anoxic depolarisation did not occur; afterwards, when the ion gradients are deteriorated, taurine may serve to exchange with other anions in order to keep extracellular glutamate low. This may continue

until taurine reached its ischemic equilibration. This together with a marked elevation of glutamate will mark the point of no return. For clinical purposes, therefore, it will be necessary to determine taurine and glutamate in parallel.

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