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Phenylsuccinate reduces KCL-induced release of GABA Evidence for the participation of the ketodicarboxylate carrier in the biosynthesis of transmitter-GABA

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Summary. The present study investigates the effects of phenylsuccinate (PS), an inhibitor of the mitochondrial ketodicarboxylate carrier (KCC), on release of γ-aminobutyric acid (GABA), glutamate (Glu), glutamine (Gln), and glycine (Gly), induced by potassium chloride (KCl) and by cardiac arrest caused by a halothane overdose. Microdialysates were collected from the hippocampus of anaesthetized rats, and analyzed by HPLC. Continuous perfusion of 50 mM PS through the dialysis probe, reduces release of GABA induced by KCl (50 mM for 10 min through the dialysis probe) by up to 72%. In addition, PS abolished KCl-induced release of Glu. Release of GABA during cardiac arrest was not reduced by PS, whereas PS reduced release of Glu in the early stage of cardiac arrest. PS furthermore increased the basal level of Gln, and reversed a decrease of Gln induced by cardiac arrest.

It is proposed that the KCC is present in GABA'ergic neurons of the rat hippocampus, and that GABA, released by KCl, can be synthesized in a KCC dependent manner. It is also suggested that ischemia-induced release of GABA, to some extent, has a non-transmitter origin. The results furthermore indicate that uptake of Gln into GABA'ergic and Glu'ergic neurons is not regulated by simple demand mechanisms.

Keywords: Amino acids – Ketodicarboxylate carrier – Phenylsuccinate – GABA – Glutamate – Glutamine – Microdialysis

Abbreviations: PS, phenylsuccinate; KCC, ketodicarboxylate carrier; GABA, γ-aminobutyric acid; Glu, glutamate; Gln, glutamine; Gly, glycine; α-KG, α-ketoglutarate; Mal, malate; KRB-buffer, Krebs-Ringer bicarbonate-buffer; HPLC, high pressure liquid chromatography.

Introduction

There is strong evidence suggesting that amino acids are involved in excitotoxic neuronal degeneration within the CNS. Much attention has been paid to amino acids of the glutamate (Glu)-family, namely Glu, aspartate (Asp), glutamine (Gln), γ-aminobutyric acid (GABA), and changes in the extracellular level of these amino acids have been shown to accompany conditions like cerebral ischemia (Benveniste et al., 1984), severe seizure-activity (Lehmann, 1987), and hypoglycemia (Sandberg et al., 1986). Amino acid extracellular homeostasis, inter/intracellular compartmentation and synthetic/metabolic pathways, are thus important issues in the interpretation of excitotoxic neurodegeneration. Using phenylsuccinate (PS) to inhibit the ketodicarboxylate carrier (KCC), evidence was recently obtained that the KCC, together with aspartate aminotransferase, is involved in the biosynthesis of transmitter-Glu (Palaiologos et al., 1988, 1989; Christensen et al., 1991). By inhibiting the KCC, PS supposedly

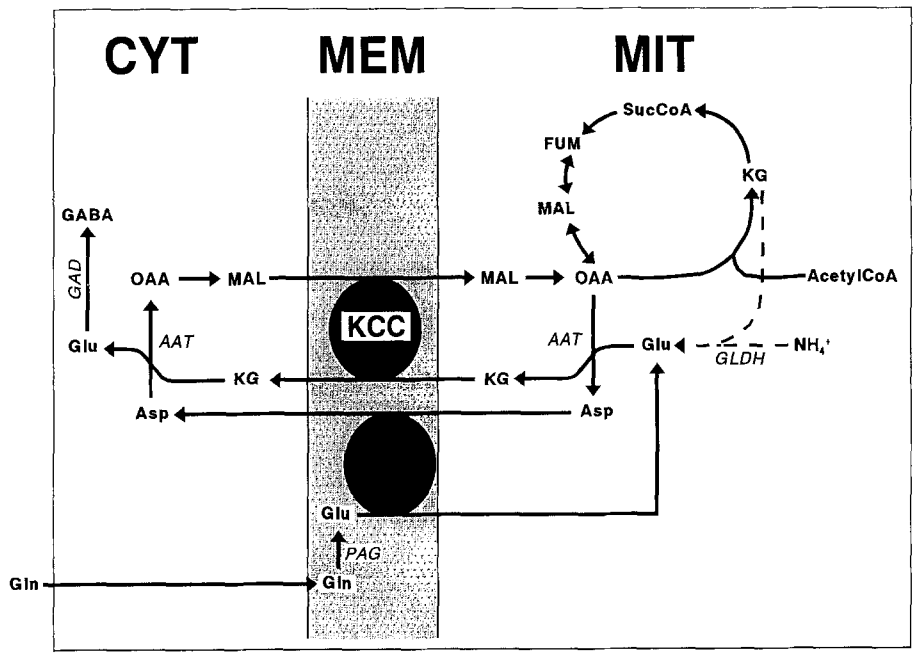


Fig. 1. Schematic representation of reactions proposed to be involved in the biosynthesis of GABA. Glu is formed from Gln in the mitochondrial membrane, as suggested by Kvamme (1983). In parallel to the synthesis of transmitter-Glu in Glu'ergic neurons (Palaiologos et al., 1988, 1989), Glu is then carried into the mitochondrial matrix in exchange with Asp. Here Glu is transaminated to α-KG by aspartate aminotransferase. By the action of the KCC, α-KG is subsequently transported across the mitochondrial membrane in exchange with Mal. In the cytosol α-KG is transaminated back to Glu by aspartate aminotransferase. The formation of GABA is accomplished by GAD acting on Glu

Abbreviations: *CYT* cytosol; *MEM* mitochondrial membrane; *MIT* mitochondrial matrix; *KCC* ketodicarboxylatecarrier; *GABA* γ-aminobutyricacid; *Glu* glutamate; *Asp* aspartate; *Gln* glutamine; *GAD* glutamate-decarboxylase; *AAT* aspartate aminotransferase; *PAG* phosphate-activated glutaminase; *GLDH* glutamate dehydrogenase; *OAA* oxaloacetate; *MAL* malate; *KG* α-ketoglutarate; FUM fumarate; *SucCoA* succinylCoA; NH_4^+ ammonia

interferes with the transport of α-ketoglutarate (α-KG) and malate (Mal) across the mitochondrial membrane (Passarella et al., 1987) (see Fig. 1 for further details). PS was previously shown not to affect release of Glu induced by 10 min of transient cerebral ischemia, indicating that such release primarily comes from non-transmitter compartments (Christensen et al., 1991). In GABA'ergic neurons, Glu functions as the immediate precursor in the biosynthesis of GABA (Reubi, 1980; Fonnum, 1981). However, it is not clear in what way Glu is provided for GABA synthesis. Presynaptic uptake of Glu into GABA'ergic terminals seems of little importance (Yu et al., 1984), but Gln is readily taken up by GABA'ergic neurons, and used as a substrate for GABA formation (Besson et al., 1981; Ward et al., 1983; Yu et al., 1984).

Using bilateral microdialysis in the rat hippocampus, we have tested the possibility of KCC participation in the biosynthesis of GABA. The KCC was inhibited by adding PS to the perfusate on one side, with the other side serving as control. Release of GABA and Glu was measured on both sides during KCl-stimulations, and during cardiac arrest induced by an overdose of halothane. Measurements of Gln were included in the study, since alterations in the extracellular level of Gln seem inversely linked to alterations in the levels of Glu and GABA (Benveniste et al., 1984; Lehmann, 1987). This linkage is thought to reflect presynaptic uptake of precursor Gln as a result of Glu- and GABA-release (Benveniste et al., 1984; Lehmann,1986). In addition, glycine (Gly), the biosynthesis of which is not related to GABA or Glu, was included in the study.

Material and methods

Five male Wistar rats (300–350 grams) were used in this experiment. Each rat was anaesthetized with 0.75% halothane in a 2:1 nitrous oxide/oxygen mixture. During the whole experiment, the rats were intubated and mechanically normoventilated. After bregma was located, bilateral holes were trepanned in the skull at points representing the coordinates of the dorsal hippocampal formation, and 2 microdialysis probes (length 2 mm and diameter 0.24 mm, CMA Microdialysis, Sweden) were bilaterally implanted. Stereotaxic coordinates in relation to bregma, with the incisor bar set at −3.3 mm beneath the interaural line, were: −3.8 mm anteroposterior, ±2.4 mm mediolateral, and 3.9 mm ventrally to the dura surface. Before sampling was commenced, and with the purpose of obtaining stable baseline levels of amino acids, the microdialysis probes were perfused with Krebs-Ringer bicarbonate (KRB)-buffer for 1.5 hours (Benveniste and Diemer, 1987). The KRB-buffer contained 122.0 mM NaCl, 3.0 mM KCl, 1.2 mM $MgSO_4$, 0.4 mM KH_2PO_4, 25.0 mM $NaHCO_3$, and, 1.3 mM $CaCl_2$, with pH = 7.40. On the right side PS (Aldrich-Chemie GmbH & Co. KG, P3,520-0), at a concentration of 50.0 mM, was added to the KRB-buffer. Based on the in vitro recoveries of molecules with similar molecular weight as PS, the extracellular concentration of PS immediately surrounding the microdialysis fiber, was estimated to be approximately 5 mM, Collin and Ungerstedt (1988). To test a possible Ca^{2+}-chelating effect of PS, the level of free Ca^{2+} in KRB-buffer and in KRB-buffer containing 5 mM PS, was measured by routine procedures, using a calcium-electrode (Radiometer, Denmark). PS was shown not to change the level of free Ca^{2+} in KRB-buffer (data not shown). During the whole experiment, the dialysis probes were perfused at a flow rate of 4 μl/min by means of microinjection pumps (CMA Microdialysis, Sweden). After sampling was commenced, dialysate was fractioned every 5 min, yielding 20 μl/sample. The experimental protocol for microdialysis was as follows: First, samples representing basal levels of amino acids were collected from both sides over a period of 20 min (4 samples). Then, 3 consecutive 10 min ON + 20 min OFF stimulations with 50.0 mM KCl (equal reduction in NaCl) were performed via

the probes, using 2 liquid switches. The extracellular K^+-level immediately surrounding the microdialysis fiber, during perfusion with 50 mM KCl, was estimated to be approximately 15 mM, Collin and Ungerstedt (1988). Twenty minutes after the third KCl-stimulation, cardiac arrest was induced by killing of the animals with an overdose of halothane (5%). The period from initiation of 5% halothane until death of the animals (registered as asystolia), never exceeded 2 min. Microdialysis was continued for an additional 20 min. At the end of the experiment, the brain was removed, frozen in isopentane at $-50°C$ for 3–5 min, and stored at $-80°C$. One or two days later, the brain was cut in 20 μm sections on a cryostat (Leitz 1720, Leica, Germany), and these were subsequently stained with hematoxyline-eosine. Histological evaluation was performed to determine the placements of the dialysis probes. The right locations of the probes in the dorsal hippocampi was verified for all 5 animals. All animal experiments were approved by the local ethical committee (Danish Animal Experiment Inspectorate).

The concentrations of GABA, Glu, Gln, and Gly, present in the samples, were analyzed by HPLC with fluorimetric detection. Injections were performed automatically, using a refrigerated autoinjector system (CMA Microdialysis, Sweden). Samples (20 μl) were derivatized with 10 μl of an o-phthaldialdehyde (Sigma P7914)/3-mercaptopropionic acid (Merck 805967)-solution, as described by Lerma and colleagues (1986). Reaction time was 60 sec. and injection volume 21 μl. The derivatized samples were separated on a 10 cm reverse-phase C^{18} nucleosil column (Knauer, Germany), using a linear gradient of methanol in a sodium acetate buffer (pH = 5.76) containing 10.0 vol% methanol and 1.3 vol% 2-propanol. Flow rate was set at 1 ml/min. Amino acids were detected by a fluorescence detector (Spectroflow 980, Kratos, USA) with excitation and emission-cutoff wavelengths set at 340 and 470 nm respectively. Concentrations of amino acids in the dialysates were quantified from peak areas (Lab Calc, Galactic Industries Corporation, USA) by means of the external standard procedure. The approximate limit of detection, based on analysis of external standards, for each amino acid was as follows: GABA and Glu, 5 nM; Gly, 10 nM; Gln, 20 nM. Differences between concentrations of amino acids related to KCl-stimulation or cardiac arrest, and those related to basal levels (average of the first 4 samples), were tested on both sides using a two-way ANOVA and Dunnett's test.

Results

GABA and glutamate

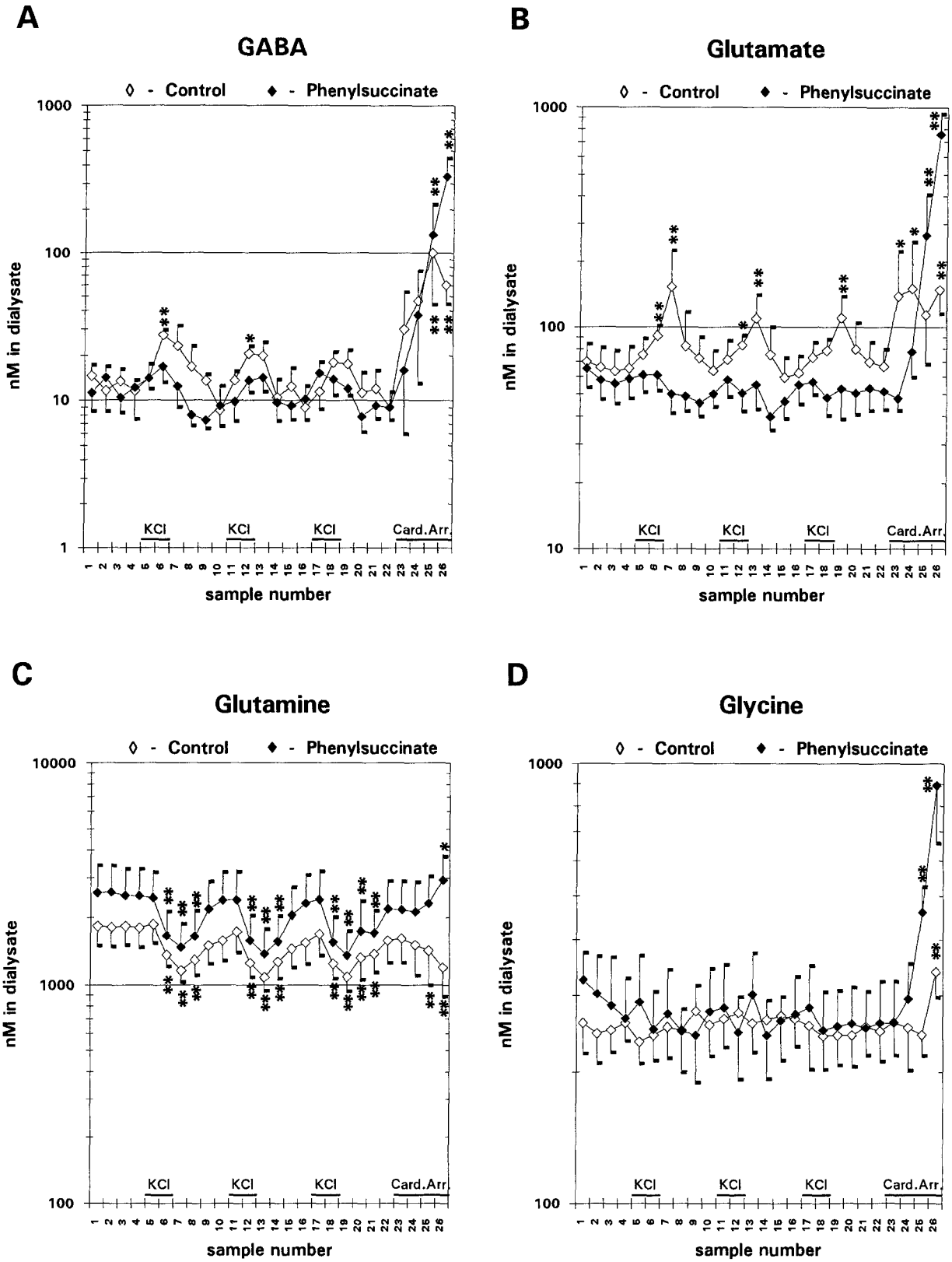
The basal levels of GABA and Glu were not changed by PS (samples 1–4). Basal dialysate concentrations of GABA, representing an average of the first 4 sample values, amounted to 13 nM and 12 nM for the control- and the PS-side respectively. Corresponding basal dialysate concentrations of Glu were 66 nM and 59 nM for the control- and the PS-side respectively (Table 1 and Fig.2A,B).

On the control-side, perfusion of the dialysis fiber with 50 mM KCl for 10 min caused the level of GABA to increase by 108% and by 62% (1st and 2nd KCl-stimulation respectively). On the PS-side, KCl resulted in minor and non-significant increases of GABA, these being 42% and 17% during the 1st and 2nd KCl-stimulation respectively (Table 1 and Fig. 2A). During the 3rd KCl-stimulation, non-significant increases (42% and 25% for the control- and the PS-side respectively) were seen on both sides. In the case of Glu, KCl increased the level of Glu on the control-side by 132%, by 65%, and by 68% during the 1st, 2nd, and 3rd KCl-stimulation respectively. On the PS-side, KCl did not give rise to any increases in the level of Glu (Table 1 and Fig. 2B).

Cardiac arrest resulted in vast increases of GABA, both on the control- and on the PS-side. During the first 15 min of cardiac arrest (samples 23–25),

Table 1. The effect of phenylsuccinate (PS) (50 mM) on dialysate concentrations (in nanomolar) ± SEM of GABA, glutamate, glutamine, and glycine during non-stimulated conditions (basal), and during potassium chloride (KCl)-stimulation (50 mM) and cardiac arrest ($N = 5$). Basal concentrations represent an average of the first 4 sample values. Concentrations representing KCl-stimulations are maximum/minimum concentrations obtained in each KCl-stimulation. Statistical significance (tested by two-way ANOVA and Dunnets tests) versus the basal concentration, is marked by * ($p < 0.05$), or by ** ($p < 0.01$)

			KCl (50 mM)			Cardiac arrest			
		Basal	1. stim.	2. stim.	3. stim.	sample 23	sample 24	sample 25	sample 26
GABA	Control	13 ± 3	27 ± 2**	21 ± 2*	18 ± 4	30 ± 23	47 ± 28	100 ± 56**	60 ± 15**
	PS	12 ± 2	17 ± 4	14 ± 3	15 ± 3	16 ± 10	37 ± 24	134 ± 83**	334 ± 109**
Glutamate	Control	66 ± 15	153 ± 72**	109 ± 31**	111 ± 27**	138 ± 84*	150 ± 97*	114 ± 46	148 ± 33**
	PS	59 ± 11	50 ± 9	55 ± 12	53 ± 11	48 ± 5	78 ± 18	266 ± 139**	756 ± 176**
Glutamine	Control	1820 ± 332	1159 ± 129**	1078 ± 139**	1090 ± 155**	1612 ± 350	1511 ± 410	1427 ± 434**	1199 ± 313**
	PS	2534 ± 803	1475 ± 389**	1376 ± 388**	1359 ± 378**	2165 ± 723	2113 ± 755	2311 ± 727	2933 ± 806*
Glycine	Control	254 ± 32	254 ± 38	274 ± 23	242 ± 39	260 ± 42	253 ± 52	243 ± 26	338 ± 42**
	PS	295 ± 64	273 ± 70	302 ± 71	250 ± 55	258 ± 64	295 ± 59	460 ± 63**	891 ± 234**


A
GABA
◊ - Control ♦ - Phenylsuccinate
1000
100
10
1
nM in dialysate
KCl
KCl
KCl
Card.Arr.
sample number
B
Glutamate
◊ - Control ♦ - Phenylsuccinate
1000
100
10
nM in dialysate
KCl
KCl
KCl
Card.Arr.
sample number
C
Glutamine
◊ - Control ♦ - Phenylsuccinate
10000
1000
100
nM in dialysate
KCl
KCl
KCl
Card.Arr.
sample number
D
Glycine
◊ - Control ♦ - Phenylsuccinate
1000
100
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dialysate concentrations of GABA increased similarly on both sides, reaching a 669% increase on the control-side and a 1017% increase on the PS-side. Whereas the level of GABA on the PS-side continued to rise during the latest recorded stage of cardiac arrest (sample 26), reaching an increase of 2683%, GABA from the control-side declined in this period to reach a level of 361% of the basal level (Table 1 and Fig. 2A). Concerning Glu, a clear side-difference was apparent during the first 10 min of cardiac arrest (samples 23–24). During this period, Glu on the control-side increased significantly by 109% and by 127% in samples 23 and 24 respectively, whereas Glu on the PS-side increased non-significantly, reaching a 31% increase in sample 24. At later stages of cardiac arrest (samples 25–26), the level of Glu on the PS-side rose more abruptly, reaching a 1175% increase in sample 26. On the control-side, Glu did not increase further, with a 124% increase in sample 26 (Table 1 and Fig. 2B).

Glutamine

The basal level of Gln tended to be elevated by PS. The basal dialysate concentration, average of samples 1–4, was 1820 nM on the control-side, and 2543 nM on the PS-side. During all 3 KCl-stimulations, the level of Gln decreased similarly on both sides, ranging from 36% to 41% on the control-side and from 42% to 46% on the PS-side. In relation to cardiac arrest, the level of Gln on the control-side decreased to a maximum of 34% in sample 26. On the PS-side the effect of cardiac arrest was reversed to an increase, with a Gln-level 16% above the basal level in sample 26 (Table 1 and Fig. 2C).

Glycine

In the case of Gly, the basal level was not altered by PS. Basal dialysate concentrations amounted to 254 nM and 295 nM for the control- and the PS-side respectively. KCl caused no alterations in the level of Gly, neither on the control- nor on the PS-side. Cardiac arrest resulted in significant increases of Gly on both sides. However, compared to a maximum increase of 33% on the

Fig. 2. The figure shows the effect of phenylsuccinate (PS) (50 mM) on release of GABA (γ-aminobutyric acid) (**A**), Glu (glutamate) (**B**), Gln (glutamine) (**C**), and Gly (glycine) (**D**), from the hippocampus of anaesthetized rats during repeated stimulation with 50 mM potassium chloride (KCl) and during cardiac arrest (Card.Arr.). Each sample represents 5 min, and sample values represent an average (±SEM) of 5 animals. The control-side is marked by hollow diamonds (◇), and the PS-side by solid diamonds (◆). Extracellular fluid was collected by microdialysis and analyzed for amino acids by HPLC. KCl and cardiac arrest was introduced as indicated by the horizontal bars. The ordinate scale represents concentration of amino acids present in the dialysate in nanomolar (*nM*). Note that the ordinate scale is logarithmic

Differences between levels related to KCl or cardiac arrest and those related to basal levels (average of the first 4 samples), were tested on both sides using a two-way ANOVA and Dunnett's test. Significance at the 1% level ($p < 0.01$) is marked by **, and at the 5% level ($p < 0.05$) by *

control-side (sample 26), the level of Gly on the PS-side increased more abruptly, reaching a 202% increase in sample 26 (Table 1 and Fig. 2D).

Discussion

The present results indicate that inhibition of the KCC with PS interferes with KCl-induced release of not only Glu, but also of GABA. The abolishment by PS of KCl-induced release of Glu agrees with previous findings that KCC-activity is essential for the synthesis of transmitter-Glu (Palaiologos et al., 1988, 1989; Christensen et al., 1991). The inhibitory effect of PS on release of Glu during the early stage of cardiac arrest (samples 23–24) has not previously been demonstrated. It is tempting to speculate that this effect of PS reflects that release of Glu in the early stage of cerebral ischemia primarily originates from the Glu transmitter-pool. A calcium-dependent release of Glu has indeed been demonstrated during the early stage of cerebral ischemia induced by decapitation (Katayama et al., 1991). During the later stages of cardiac arrest (samples 25–26), release of Glu on the PS-side increased to reach a level more than 20 times that of the control-side. It is difficult to interpret in what way PS stimulates release of Glu. There is evidence that ischemia-induced release of Glu originates not only from the transmitter-pool, but also from a metabolic pool, possibly located in astrocytes (Kauppinen et al., 1988; Christensen et al., 1989). It seems possible that PS, by reducing the synthesis of transmitter-Glu/GABA, induces a decrease in the demand for precursor Gln. In agreement with this, PS increased the extracellular level of Gln. An increased extracellular level of Gln might lead to a decrease in astrocytic synthesis of Gln from Glu, and more metabolic Glu would thus be accessible for release during cardiac arrest. It must be underlined that this interpretation needs further investigation for its validation. In a previous study, using the 4-vessel occlusion model of transient cerebral ischemia, we found no effects of PS on ischemia-induced release of Glu (Christensen et al., 1989). These seemingly contradictory results regarding the effects of PS on release of Glu during cerebral ischemia, might be attributed to differences between the models of cerebral ischemia.

The reduction by PS of KCl-induced release of GABA provides evidence that the KCC is present in GABA'ergic neurons of the rat hippocampal formation, and that KCC activity is important for the synthesis of GABA released by KCl. It follows that inhibition of the transport of α-KG/Mal across the mitochondrial membrane interferes with the formation of transmitter-GABA. Since Glu is the immediate precursor of GABA, it is possible that the inhibitory effect of PS on the release of GABA, is accomplished in a way similar to the inhibitory effect of PS on the release of Glu,- through a blockade of the complex, partly intramitochondrial, transamination pathway (Fig. 1). There is evidence that in GABA'ergic neurons, a transport exists across the mitochondrial membrane of substances needed for GABA formation in the cytosol. The labelling pattern of GABA, after exposure of the intact rat brain to [^{13}C]glucose, provides direct evidence for GABA formation via pyruvate carboxylation (Brainard et al., 1989). This demonstrates that, in GABA'ergic neurons there is a metabolic connection between a mitochondrial pool of TCA (tricarboxylic acid)-cycle constituents,

and the Glu precursor pool used for synthesis of GABA. Synthesis of precursor Glu through transamination requires the presence of aspartate aminotransferase in hippocampal GABA'ergic neurons, both intramitochondrially, and in the cytosol. Aspartate aminotransferase-like immunoreactivity has been identified in GABA'ergic neurons of different brain structures (Wenthold and Altschuler, 1983; Kamisaki et al., 1984), but it is not known if this applies also to GABA'ergic neurons of the rat hippocampal formation. Studies on cultured GABA'ergic neurons do not support the formation of GABA in a manner dependent on aspartate aminotransferase-activity. Unlike Glu'ergic neurons, GABA'ergic neurons in vitro do not readily utilize exogenously applied α-KG or Mal for transmitter synthesis (Hertz et al., 1992). Of interest in this respect is the proposal by Hertz and colleagues (1992) that at least 2 different subpopulations of mitochondria exist in GABA'ergic neurons, both of which are accessible only to certain substrates. This could imply that GABA can be synthesized by different metabolic pathways, and explain the inability of PS to reduce KCl-induced release of GABA completely.

An ion-chelating effect has been ascribed to compounds like succinate. It is however unlikely that a Ca^{2+}-chelating effect of PS can explain its effects on KCl-induced release of GABA and Glu, since the level of free Ca^{2+} in KRB-buffer perfusate was unaltered after 5 mM PS (estimated extracellular concentration of PS immediately surrounding the microdialysis fiber) had been added (data not shown). In agreement with this, in superfusion of cerebellar granule cells, Ca^{2+}-dependent, KCl-stimulated [^{3}H]D-aspartate-release was not reduced in the presence of 5 mM PS (Palaiologos et al., 1989). In solving the problem of a possible Ca^{2+}-chelating effect of PS, a more direct approach would undoubtedly be to measure the influence of PS on KCl-induced release of neurotransmitters not releated to the Glu-family, e.g. glycine (in the spinal cord), or dopamine. Such data is however, not available at present.

Concerning cardiac arrest, release of GABA was not reduced by PS. During the first 15 min after cardiac arrest (samples 23–25), vast GABA-increases of similar magnitude were seen on the 2 sides. Taking into consideration that PS reduced KCl-induced release, this indicates that ischemia-induced release of GABA, at least in part, originates from non-transmitter compartments. In the latest sample after cardiac arrest (sample 26), release of GABA continued to rise on the PS-side, reaching a level more than 5 times that of the control-side. A PS-induced accumulation of astrocytic GABA, based on mechanisms similar to those described for PS-induced accumulation of astrocytic Glu (see above), could be used to interpret this effect of PS. However, since data on the cellular origin of ischemia-induced release of GABA is sparse, this observation must remain to be clarified in future studies.

In relation to Gln, it was previously demonstrated that KCl-stimulation (Nadler et al., 1977), as well as cerebral ischemia (Benveniste et al., 1984), produce a decrease in the extracellular level of this Glu/GABA-precursor. These effects were reproduced on the control-side in our experiment. Comparing basal levels of the 2 sides, there was a tendency of PS to increase the basal level of Gln. This effect may reflect a decrease in the demand for precursor Gln in Glu'ergic and GABA'ergic neurons. In relation to KCl-stimulation, Gln-decreases of similar

magnitude were found on the 2 sides. This observation speaks against the existence of a tight coupling between neuronal Glu/GABA-release and Gln-uptake. It indicates that KCl, or depolarization, per se, either stimulates Gln-uptake into Glu'ergic and/or GABA'ergic terminals, or interferes with Gln-release from astrocytes, regardless of impaired neuronal Glu/GABA-release. During cardiac arrest, the decrease of Gln seen on the control-side was reversed to an increase on the PS-side. It is tempting to speculate that Gln taken up presynaptically, was not converted to Glu/GABA because of the effect of PS, and that during cardiac arrest such Gln was released extracellularly.

With respect to Gly, the level of this amino acid was not subjected to any changes during KCl-stimulation, neither on the control-, nor on the PS-side. These observations agree with Gly being a non-transmitter in the rat hippocampus, and that KCC-activity is not involved in the biosynthesis of Gly. During cardiac arrest, elevated levels of Gly were detected on both sides. However, on the PS-side Gly rose more abruptly, reaching a level more than 2 times that of the control-side. A tenable interpretation of this observation needs further investigation.

It must be considered that the effects of cardiac arrest on the release of amino acids could be influenced by the high concentration of halothane used to induce cardiac arrest. No data exist regarding the effects of high concentrations of halothane on the extracellular release of amino acids. At concentrations below 2%, the anaesthetic effect of halothane is, in part, ascribed to a depressant action at excitatory synapses, possibly through a reduction in presynaptic transmitter-synthesis and/or transmitter-release. Exposure to concentrations of 3–4%, over a period of 30 min, has been shown to increase the content of Glu and Gly in whole rat brain, without affecting the content of GABA (Arai et al., 1990). In the present experiment the time period from initiation of 5% halothane until cardiac arrest never exceeded 2 min. It seems unlikely that such short exposure to high concentrations of halothane, to any great extent, would influence amino acid metabolism. It is possible that 5% halothane initially affects the release of amino acids induced by cardiac arrest, however, the pattern and magnitude of amino acid-release obtained in our model, is comparable with that obtained in other models of cerebral ischemia (Benveniste et al., 1984; Korf et al., 1988).

In summary, we have shown that inhibition of the KCC by PS potently inhibits KCl-induced release of not only Glu, but also of GABA. It is proposed that Glu, destined for GABA formation, can be synthesized in a KCC dependent manner, similar to that seen in Glu'ergic neurons in the synthesis of transmitter-Glu. Furthermore, release of GABA during cerebral ischemia is, at least partly, unrelated to the transmitter-pool. Finally, uptake of precursor-Gln into Glu'ergic and GABA'ergic neurons seems not to be the result of simple demand mechanisms governed by release of transmitter-Glu/GABA.

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