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Effects of uptake carrier blockers SK&F 89976-A and L-*trans*-PDC on in vivo release of amino acids in rat hippocampus

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Abstract

This report describes the in vivo effects of the uptake carrier blockers 1-(4,4-diphenyl-3-butenyl)-3-piperidine carboxylic acid hydrochloride (SK&F 89976-A) and L-*trans*-pyrrolidine-2,4-dicarboxylate (L-*trans*-PDC) on basal and K^+-evoked extracellular levels of γ-aminobutyric acid (GABA), glutamate, aspartate and taurine in the hippocampus of anaesthetised rats, using the microdialysis technique. SK&F 89976-A increased extracellular GABA levels under K^+-depolarised conditions and did not affect extracellular glutamate, aspartate and taurine levels, indicating its selective effect on GABA uptake. L-*trans*-PDC dose dependently increased basal and K^+-evoked extracellular glutamate levels, and did not affect extracellular GABA levels, but increased basal aspartate and taurine levels. The K^+-evoked release of GABA and glutamate, measured in the presence of both SK&F 89976-A and L-*trans*-PDC, was Ca^{2+}-dependent for about 50% and 65%, respectively. In contrast, the release of the putative amino acid transmitters aspartate and taurine was not Ca^{2+}-dependent. These results indicate that (1) in rat hippocampus uptake carriers actively regulate extracellular GABA and glutamate levels, (2) the GABA and glutamate released by K^+ was derived from both Ca^{2+}-dependent (presumably vesicular) and Ca^{2+}-independent (presumably cytosolic) pools, whereas aspartate and taurine release was exclusively from Ca^{2+}-independent pools.

Keywords: Microdialysis; Hippocampus; Amino acid; Uptake carrier; Vesicular release; Ca^{2+} dependency

1. Introduction

The uptake of neurotransmitters via carriers is an important mechanism in the regulation of extracellular amino acid levels in the brain. These carriers rapidly remove γ-aminobutyric acid (GABA) and glutamate from the synaptic cleft, thus avoiding excessive receptor stimulation and thereby keeping neurones responsive to subsequent stimulation (Attwell and Mobbs, 1994). The hippocampal formation is an intensively studied brain region with respect to changes in synaptic transmission of GABA and glutamate in several processes such as long-term potentiation (Bliss and Collingridge, 1993), epilepsy (Kamphuis and Lopes da Silva, 1990) and ischaemia (Globus et al., 1991). In vivo studies of the release of these transmitters in the hippocampus have been performed mainly in experimental models of ischemia and hypoglycaemia, using the microdialysis technique. In these studies massive increases in GABA and glutamate, but also in non-transmitter amino acids, were observed (Andiné et al., 1991; Globus et al., 1991). Whether persistent changes in extracellular amino acid levels can be detected in vivo during long-term potentiation is still a matter of debate (Bliss et al., 1986; Aniksztejn et al., 1989; Ghijsen et al., 1992). Since the time resolution of in vivo microdialysis is rather poor, local changes in extracellular GABA and glutamate levels may be rapidly cleared by their uptake carriers without being monitored by the dialysis probe. For this reason uptake carrier blockers are often used in microdialysis studies (Westerink and De Vries, 1989; Fink-Jensen et al., 1992; Sved and Curtis, 1993). In contrast to other brain regions, application of these blockers in hippocampal microdialysis studies is rather scarce. In a few studies dihydrokainate, a relatively weak glutamate uptake inhibitor, has been used (Muñoz et al., 1987; Millan et al., 1991). Recently, L-*trans*-pyrrolidine-2,4-dicarboxylate (L-*trans*-PDC) (Bridges et al., 1991) was shown to be a highly selective and potent glutamate uptake inhibitor in rat hippocampus synaptosomes (Mitrovic and Johnston, 1994). Although GABA uptake inhibitors, such as the substrate blocker nipecotic acid and the potent inhibitor 1-(4,4-di-

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phenyl-3-butenyl)-3-piperidine carboxylic acid hydrochloride (SK&F 89976-A) (Yunger et al., 1984), have been used both in vitro and in vivo, the application of these compounds in hippocampus studies in vivo has not been reported yet.

In the present study we addressed the question to what extent the release of GABA and glutamate is influenced by uptake carrier activity. We investigated the effects of the GABA uptake carrier blocker SK&F 89976-A and the glutamate uptake carrier blocker L-*trans*-PDC on extracellular amino acid levels measured in vivo in the hippocampus of the rat. The exocytotic (vesicular) part of changes in extracellular GABA and glutamate levels was estimated by determining its Ca^{2+} dependence and compared it to that of the putative amino acid transmitters, aspartate and taurine. We used the microdialysis technique to determine basal levels and K^+ depolarisation-dependent releasable pools of hippocampal endogenous amino acids. To decrease fluctuations related to changing neuronal activity, microdialysis was carried out in anaesthetised animals. Part of this study has been presented in abstract form (Zuiderwijk et al., 1993).

2. Materials and methods

2.1. Materials

SK&F 89976-A was kindly provided by Dr. Skidmore (Smith, Kline & French Research, Welwyn, England). L-*trans*-PDC was purchased from Tocris Neuramin (Bristol, UK). Chloral hydrate and lidocaine were from Merck (Amsterdam, Netherlands) and urethane was from De Onderlinge Pharmaceutische Groothandel (Utrecht, Netherlands). Hypersil was from Shandon (L.C. Service, Emmen, Netherlands). All other chemicals were of the purest grade available and were obtained from Sigma (Brunschwig, Amsterdam, Netherlands) and Merck (Amsterdam, Netherlands).

2.2. Microdialysis probes and in vitro recoveries

2.2.1. Microdialysis probes

Concentric dialysis probes were constructed in our laboratory following the method of Santiago and Westerink (1990) with a slight modification. The dialysis membrane (OD 0.23 mm, Asahi AM-160 Nova from Cablon Medical, Leusden, Netherlands) was sealed with Araldit epoxy glue except for a 2.0 mm long section of membrane exposed at the tip.

2.2.2. In vitro recoveries

In vitro recoveries of GABA, glutamate and K^+ were determined in order to estimate their extracellular concentrations. Probes were perfused with artificial cerebrospinal fluid (a-CSF) at 2.0 μl/min. GABA and glutamate recoveries were determined by placing the probes in a-CSF solution containing 10 μM of both amino acids; 20 min samples were collected and analysed by high-performance liquid chromatography (HPLC). The recovery was expressed as the percentage of amino acid passing across the membrane and was calculated as $(c_{in}/c_{out}) \times 100\%$, with c_{out} being the amino acid concentration in the solution surrounding the probe and c_{in} being the amino acid concentration in the collected samples. The K^+ recovery was determined by placing the probes in a normal a-CSF solution and perfusing them with a-CSF containing 100 mM K^+. The recovery was defined as the percentage of K^+ passing across the membrane from the inside to the outside of the probe and was calculated as $(c_{in} - c_r) \times 100\%$, with c_{in} being the initial K^+ concentration in the perfusion fluid and c_r being the remaining K^+ concentration in the collected samples. K^+ concentrations were measured using an atomic absorption spectrophotometer (Perkin Elmer). Experiments were carried out at room temperature. In vitro recoveries were: $8.9 \pm 0.4\%$ for GABA, $7.6 \pm 0.1\%$ for glutamate and $28.6 \pm 2.0\%$ for K^+ (mean ± S.E.M., $n = 3$).

2.3. Animals

Male Wistar rats (200–350 g body weight) were housed in groups in Plexiglas cages on a 12 h dark/12 h light schedule with free access to food and water. The animals were housed individually the day before surgery. All experimental procedures were controlled and approved (June 1992) by the Animal Experiments Committee of the Faculties of Biology and Chemistry, University of Amsterdam.

2.4. Probe implantation and in vivo microdialysis

2.4.1. Probe implantation

The animals were anaesthetised with chloral hydrate (40 mg/100 g body weight, i.p.) and mounted in a stereotaxic frame (tooth bar 5.0 mm above interaural line (Pellegrino et al., 1979)) with lidocaine (5%) applied to the ear bars. Dialysis probes filled with a-CSF were implanted in the left dorsal hippocampus (A/P 2.4 mm, M/L 1.4 mm, D/V 3.4 mm) with the dialysis membrane covering the entire extent of the CA1-dentate gyrus field. Probes were fixed to the skull with dental acrylic cement. The rats were allowed to recover for 1 day before microdialysis was started. Initial studies were performed directly after probe implantation in urethane-anaesthetised animals. Under these circumstances, the effects of K^+ depolarisation, especially on glutamate perfusate concentrations, were highly variable (data not shown).

2.4.2. In vivo microdialysis

20–24 h after implantation, the rats were anaesthetised with urethane (0.15 g/100 g body weight, i.v.) and placed

in the stereotaxic frame. Probes were perfused with a-CSF containing (in mM) 150 NaCl, 3 KCl, 2 $CaCl_2$, 5 Hepes, pH 7.4, at a rate of 2.0 μl/min, using a syringe-type infusion pump (Harvard Apparatus). After a stabilisation period of 1.5 h, 20 min (40 μl) samples were collected with an automated sample collector (Gilson) and kept cool (at 2°C) during the experiment by means of a recirculation cooler (Frigomix). K^+ depolarisation was induced by raising the potassium concentration to 100 mM for 1 h (corrected for osmolarity by replacing NaCl by KCl).

The effects of the uptake carrier blockers were studied in separate experiments with either SK&F 89976-A (0.1 and 0.5 mM) or L-*trans*-PDC (0.2 and 1.0 mM) in the perfusion medium. Ca^{2+} dependence studies were performed in the presence of both 0.5 mM SK&F 89976-A and 1.0 mM L-*trans*-PDC throughout the dialysis experiment. Perfusion was carried out with five different solutions which were changed every hour and contained (1) basal a-CSF with Ca^{2+}; (2) basal Ca^{2+}-free a-CSF; (3) Ca^{2+}-free a-CSF with 100 mM K^+; (4) as in (2) basal Ca^{2+}-free a-CSF; (5) a-CSF with Ca^{2+} and 100 mM K^+. Ca^{2+}-free medium contained 10 mM Mg^{2+} and 2 mM EGTA. A liquid switch with a zero dead volume was used to switch between the different perfusion fluids. After the experiment, collected samples were stored at −20°C in the presence of 1% (w/v) trichloroacetic acid and 0.5 μM L-homoserine, as external standard, for amino acid content determination. Rats were killed by an overdose of pentobarbital immediately after the last sample had been collected and fixed by perfusion of the ascending aorta with 4% paraformaldehyde, 0.05% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4). The brain was then removed and sectioned for histological verification of probe placement.

2.5. Quantification of amino acid concentrations

Stored microdialysis samples (40 μl) were centrifuged for 4 min (12 000 × *g*) and 30 μl supernatant was used for analysis. Amino acid content was quantified by high-per-

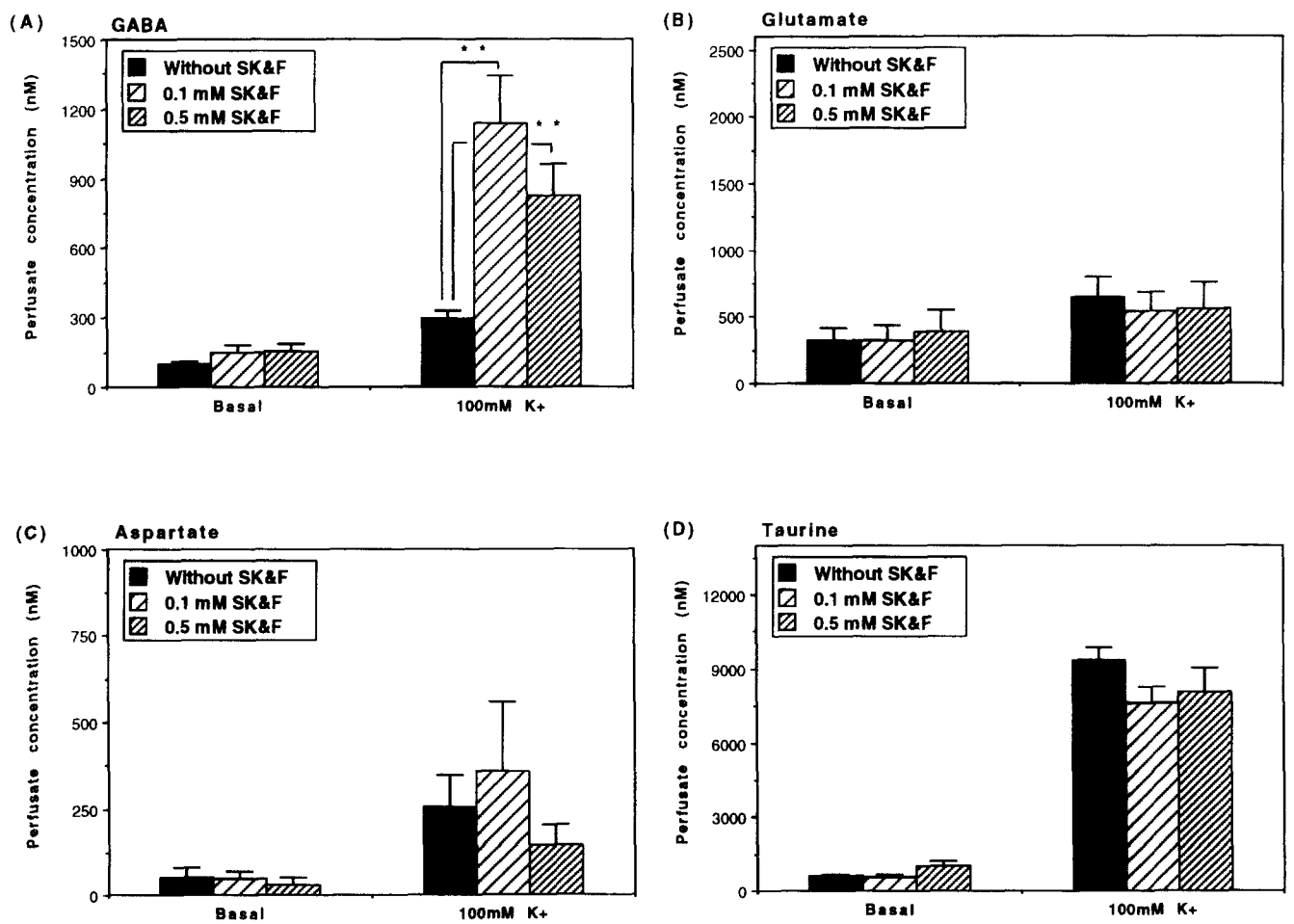

Fig. 1. Effects of SK&F 89976-A on basal and K^+-stimulated GABA, glutamate, aspartate and taurine levels, measured 20–24 h after probe implantation. Data are presented as mean basal plateau values ± S.E.M. and mean K^+-stimulated peak values ± S.E.M. ($n = 4–8$). Comparisons were made between mean basal values and corresponding K^+-stimulated peak values, which were significantly different in all cases ($P < 0.05$) (not indicated in graph), and between different SK&F 89976-A concentrations under the same conditions (indicated by connection bars), using Wilcoxon-Mann-Whitney two-sample test: ** $P \leq 0.01$.

formance liquid chromatography (HPLC) after precolumn derivatisation of a 25 μl sample with 50 μl *o*-phthalaldehyde. The 12.5 cm long, 4.6 mm diameter column packed with Hypersil C-18 (3 μm particle size), was eluted isocratically with a buffer containing 0.1 M Na_2HPO_4, 1 mM Na_2-ethylenediaminetetraacetate (EDTA), 0.3% tetrahydrofuran and 35% (v/v) HPLC-grade methanol. The *o*-phthalaldehyde derivates were detected by a Jasco (model F821-FP) fluorimeter (detection limits 20 fmol for GABA and 10 fmol for glutamate) and evaluated on a Spectra Physics integrator. Neither SK&F 89976-A nor L-*trans*-PDC interfered with the chromatograms. Amino acid levels were calculated by comparison with a standard mixture of 1.0 μM of these amino acids, as described previously (Verhage et al., 1989).

2.6. Data presentation and statistics

All data are presented as mean values ± S.E.M. of *n* independent experiments. Statistical analysis was performed using the Wilcoxon-Mann-Whitney two-sample test, or the Wilcoxon signed rank test, as indicated. Values were considered significantly different when $P < 0.05$.

3. Results

3.1. Uptake inhibition

The effects of the GABA uptake inhibitor SK&F 89976-A and the glutamate uptake inhibitor L-*trans*-PDC on basal and high K^+-evoked amino acid levels were investigated 20–24 h after probe implantation. Basal perfusate concentrations were stable over a 2 h period in all experiments, regardless of whether uptake inhibitors were present in the medium or not (data not shown). Mean basal plateau values were calculated. During 1 h of high K^+ a transient increase was observed and the peak value (which

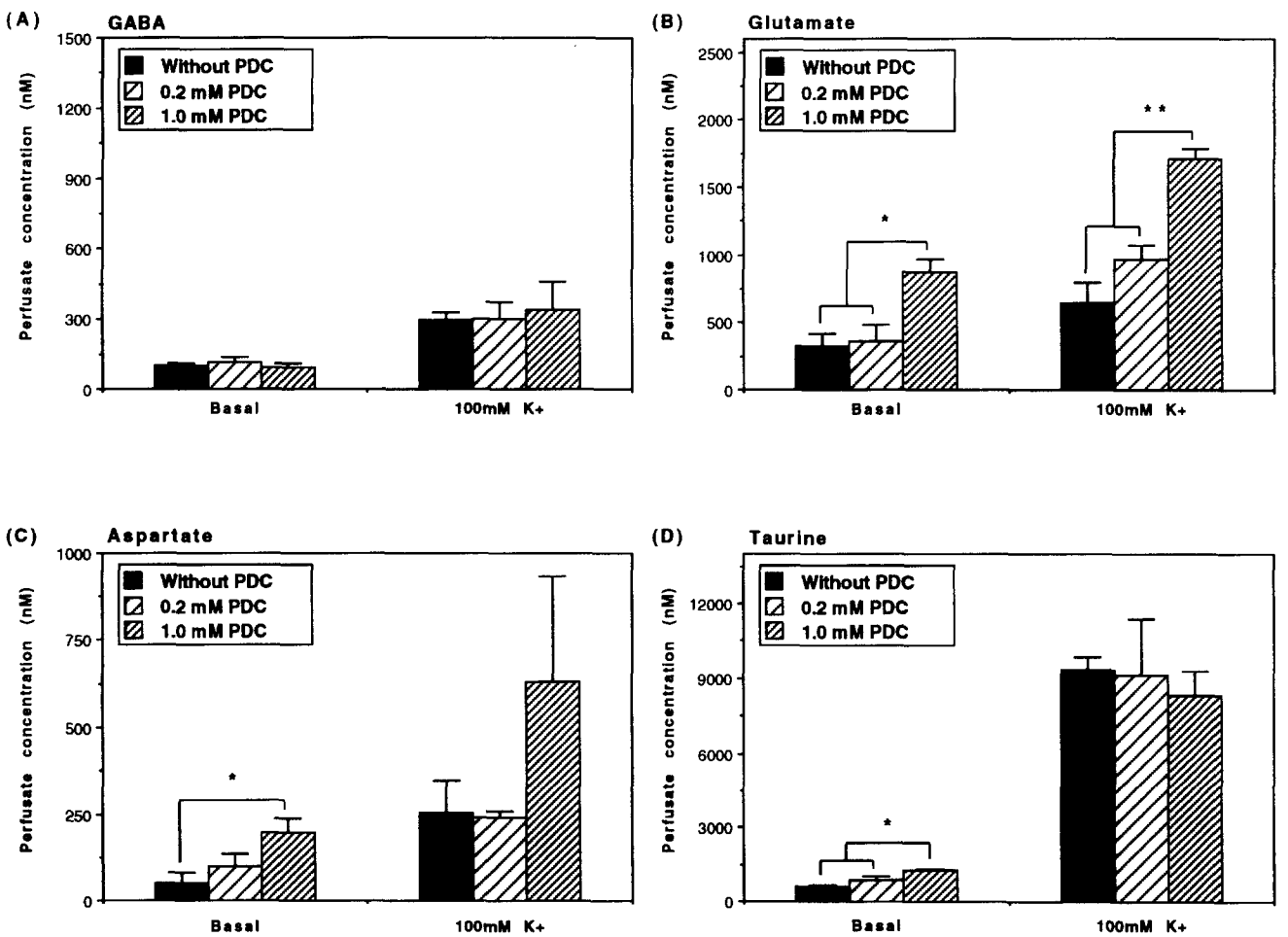

Fig. 2. Effects of L-*trans*-PDC on basal and K^+-stimulated GABA, glutamate, aspartate and taurine levels, measured 20–24 h after probe implantation. Data are presented as mean basal plateau values ± S.E.M. and mean K^+-stimulated peak values ± S.E.M. ($n = 4–8$). Comparisons were made between mean basal values and corresponding K^+-stimulated peak values, which were significantly different in all cases ($P < 0.05$) (not indicated in graph), and between different L-*trans*-PDC concentrations under the same conditions (indicated by connection bars), using Wilcoxon-Mann-Whitney two-sample test: * $P < 0.05$, ** $P \leq 0.01$.

was reached in the first, second, or third fraction) was taken to show the maximal K^+-evoked effect.

3.1.1. Effects of SK&F 89976-A

The basal GABA perfusate concentration (98 ± 13 nM) was not significantly increased in the presence of either 0.1 or 0.5 mM SK&F 89976-A (Fig. 1A). In the absence of SK&F 89976-A the K^+-induced release of GABA was 3-fold higher than basal levels. In the presence of 0.1 and 0.5 mM SK&F 89976-A, this increase was 11-fold and over 8-fold (not significantly different from each other), respectively. In the absence of SK&F 89976-A basal levels of glutamate (323 ± 96 nM), aspartate (51 ± 30 nM) and taurine (590 ± 76 nM) were increased by high K^+ by 2-fold, 5-fold and 16-fold, respectively. Neither 0.1 nor 0.5 mM SK&F 89976-A had significant effects on the basal and K^+-evoked levels of these amino acids (Fig. 1B, C and D).

3.1.2. Effects of L-trans-PDC

The basal glutamate concentration was not significantly increased in the presence of 0.2 mM L-*trans*-PDC, but was increased almost 3-fold in the presence of 1.0 mM L-*trans*-PDC (Fig. 2B). The 2-fold increase in glutamate release elicited by high K^+ in the absence of L-*trans*-PDC was elevated to 3-fold and over 5-fold in the presence of 0.2 and 1.0 mM L-*trans*-PDC, respectively. The K^+-evoked glutamate release in the absence of L-*trans*-PDC increased almost 3-fold after addition of 1.0 mM L-*trans*-PDC.

Neither 0.2 nor 1.0 mM L-*trans*-PDC had any effects on the basal and K^+-evoked levels of GABA (Fig. 2A).

The basal aspartate and taurine concentrations in the absence of L-*trans*-PDC were increased in the presence of 1.0 mM L-*trans*-PDC by 4-fold and 2-fold, respectively (Fig. 2C and D). No significant effects of L-*trans*-PDC on the K^+-evoked levels of aspartate and taurine were observed.

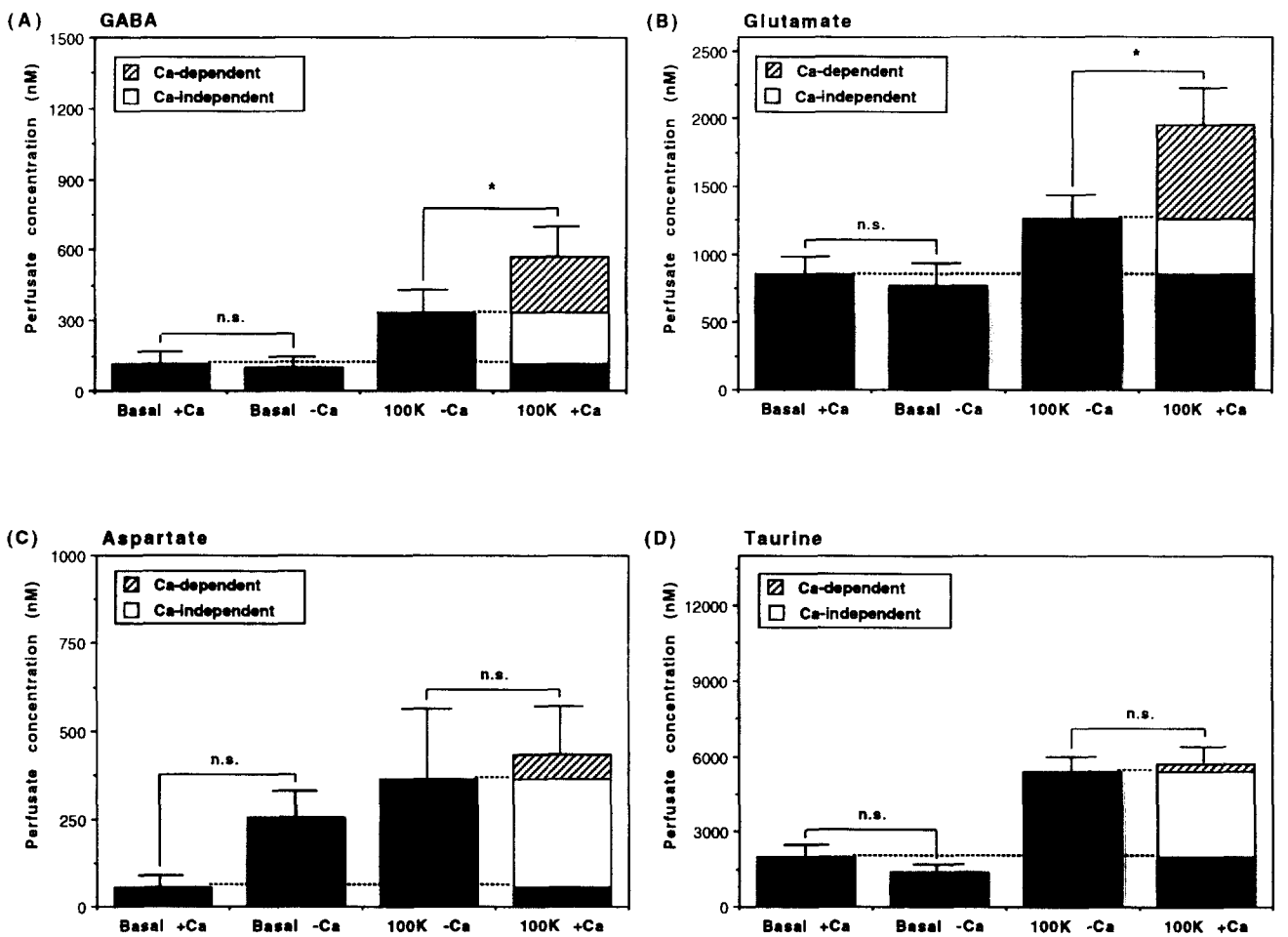

Fig. 3. Effects of calcium on basal and K^+-stimulated GABA, glutamate, aspartate and taurine levels, measured 20–24 h after probe implantation. All experiments were performed in the presence of both 0.5 mM SK&F 89976-A and 1.0 mM L-*trans*-PDC. Data are presented as mean basal plateau values ± S.E.M. and mean K^+-stimulated peak values ± S.E.M. ($n = 6$). Repeated stimulation with high K^+ (at 1 h intervals) was done in the absence and presence of Ca^{2+} in the perfusion fluid, respectively. In each graph, the error bar on the subdivided last column represents the S.E.M. of the total value. Comparisons between the absence and presence of Ca^{2+} of basal plateau values and K^+-stimulated peak values were made by using Wilcoxon signed rank test: * $P \leq 0.014$. The Ca^{2+}-dependent fraction was obtained by subtracting the mean K^+-stimulated peak value without Ca^{2+} from the mean K^+-stimulated peak value with Ca^{2+}. The Ca^{2+}-independent fraction was obtained by subtracting the Ca^{2+}-dependent component from the total release. The latter was defined as the K^+-stimulated peak value minus the basal plateau value, both in the presence of Ca^{2+}.

3.2. Ca^{2+}-dependent release

As the increased concentration of both GABA and glutamate in the extracellular fluid may originate from neuronal and non-neuronal pools, the Ca^{2+} dependence of the basal and high K^+-evoked amino acid levels was determined. In addition, extracellular levels of the amino acids aspartate and taurine were measured for comparison. Since there were no cross-effects, neither of SK&F 89976-A on glutamate release nor of L-*trans*-PDC on GABA release (Figs. 1 and 2), all experiments were performed in the presence of both 0.5 mM SK&F 89976-A and 1.0 mM L-*trans*-PDC. The Ca^{2+}-free perfusion medium contained 10 mM Mg^{2+} (to block Ca^{2+}-channel activity) and 2 mM EGTA (to chelate residual Ca^{2+}). To compare glutamate and GABA levels in the presence of Ca^{2+} with those in the absence of Ca^{2+}, microdialysis under basal and high K^+ conditions was performed by alternating perfusion with Ca^{2+}-containing and Ca^{2+}-free media (see Materials and Methods). Whether time effects or depletion of amino acid stores by repeated stimulation with high K^+ occurred in the Ca^{2+}-dependence protocol was checked in experiments in which high K^+ was applied repeatedly without changing Ca^{2+}. No significant differences in extracellular levels of the amino acids measured were observed between both stimulations with high K^+ in the presence of Ca^{2+} (tested by Wilcoxon-Mann-Whitney two-sample test).

Basal perfusate concentrations of the amino acids analysed were stable, regardless of whether calcium was present in the medium (data not shown). Mean basal plateau values were calculated. During high K^+ perfusion (first in the absence, second in the presence of Ca^{2+}), transient increases were observed. The highest values were taken to indicate the maximal K^+-evoked effect.

Under basal conditions no significant changes in GABA and glutamate concentrations were observed when Ca^{2+} was removed (Fig. 3A and B). For aspartate and taurine (Fig. 3C and D) the changes in basal levels were larger, but not significant ($P = 0.09$ for aspartate, $P = 0.07$ for taurine, Wilcoxon signed rank test).

For both GABA and glutamate, the first K^+-stimulated peak value, in the absence of Ca^{2+}, was significantly lower than the second K^+-stimulated peak value, in the presence of Ca^{2+} (Fig. 3A and B). This difference corresponds to the Ca^{2+}-dependent release component, which accounted for 50% of the total GABA release and 65% of the total glutamate release (Fig. 3A and B, last bars).

For aspartate and taurine no significant differences between K^+-stimulated peak values with and without Ca^{2+} were observed (Fig. 3C and D).

4. Discussion

In the present study we investigated the effects of the uptake carrier blockers SK&F 89976-A and L-*trans*-PDC on in vivo basal and K^+-evoked extracellular levels of GABA, glutamate, aspartate and taurine measured in the hippocampus of anaesthetised rats. The main findings are: (1) SK&F 89976-A increased extracellular GABA levels under K^+-depolarised conditions, but not under basal conditions, and showed no cross-effects on extracellular glutamate, aspartate and taurine levels, indicating its selective effect on GABA uptake. (2) L-*trans*-PDC dose dependently increased both basal and K^+-evoked extracellular glutamate levels and did not influence extracellular GABA levels, but increased basal aspartate and taurine levels. (3) The depolarisation-evoked release of GABA and glutamate, measured in the presence of both uptake blockers, was derived from two releasable pools: a Ca^{2+}-dependent, presumably vesicular pool of neurones, and a Ca^{2+}-independent, presumably cytosolic pool of neurones and/or glial cells. In contrast, the release of the amino acids aspartate and taurine was not Ca^{2+}-dependent, indicating that release was from the cytosolic pool only.

4.1. Role of uptake carriers in regulating extracellular amino acid concentrations

Our results indicate a highly active regulation of extracellular glutamate and GABA levels in rat hippocampus by their uptake carriers, since an increase in extracellular glutamate and GABA was elicited by L-*trans*-PDC and SK&F 89976-A, respectively. Basal dialysate concentrations of glutamate and GABA, in the absence of the uptake blockers (about 300 and 100 nM, respectively), were similar to earlier reported values in rat hippocampus (Andiné et al., 1991; Globus et al., 1991). Assuming that the in vivo recoveries for both amino acids are similar to those found in vitro (see Section 2), the carrier-regulated extracellular glutamate and GABA levels amount to 4 and 1 μM, respectively. These levels are within the range of the affinities of the uptake carriers for glutamate (2–50 μM) and GABA (0.8–20 μM), as reported for different transporters investigated in situ or after functional expression of different clones (Keynan and Kanner, 1988; Lopez-Corcuera et al., 1992; Kanai et al., 1994).

SK&F 89976-A did not have any effect on basal GABA levels, indicating that the GABA transporter is not active under polarised conditions. In contrast, L-*trans*-PDC caused a dose-dependent increase of basal glutamate levels. This is probably due to the fact that at higher concentrations L-*trans*-PDC can act as a transportable substrate of the glutamate uptake carrier (Griffiths et al., 1994), thereby inducing additional glutamate release from a cytosolic pool by heteroexchange. This is supported by the result that basal glutamate levels in the presence of 1.0 mM L-*trans*-PDC were completely Ca^{2+}-independent (Fig. 3B). For SK&F 89976-A, which in our study had similar effects to L-*trans*-PDC only under K^+ depolarisation, no such transportable properties have been described. It is believed that

SK&F 89976-A blocks the GABA uptake carrier only at the extracellular side (Larsson et al., 1988). The effects of SK&F 89976-A were highly specific for GABA, indicating that only GABA uptake is blocked. L-*trans*-PDC also influenced basal aspartate levels, which may be explained by heteroexchange induced by it acting as a substrate of the aspartate/glutamate carrier. In addition, L-*trans*-PDC also influenced basal taurine levels, which may be the result of activation of glutamate transporters in neuronal and glial cells. Such an activation would cause a small depolarisation of these cells, leading to the release of taurine. The fact that under K^+ depolarisation no effects of L-*trans*-PDC on aspartate and taurine were observed may be explained by the low activity of the Na^+-dependent high-affinity uptake carriers, due to a strong reduction of the electrochemical gradient for Na^+ under this condition (Erecinska, 1987; Nicholls, 1989).

In a few other microdialysis studies in rat hippocampus dihydrokainate was used as glutamate uptake blocker, although not in combination with high K^+ or Ca^{2+} withdrawal (Muñoz et al., 1987; Millan et al., 1991). More recently, in one study the more potent blocker L-*trans*-PDC was used, and a slightly lower increase in basal glutamate levels was observed than in our study, possibly due to different experimental conditions since the measurements in that study were done in freely moving rats (Millan et al., 1993). In contrast to glutamate uptake blockers, the effects of SK&F 89976-A or other GABA uptake blockers on high K^+-evoked GABA levels in the hippocampus in vivo have not been reported before.

4.2. Amino acid release from different intracellular pools

4.2.1. GABA and glutamate

Since we found that the uptake carriers were highly active in regulating extracellular glutamate and GABA levels even under K^+-depolarised conditions, which can mask their detection, Ca^{2+}-dependence studies were performed in the presence of both uptake carrier blockers. Our results reveal that about 50% of the K^+-induced GABA release and approximately 65% of the glutamate release was mediated by Ca^{2+}-dependent mechanisms, which is in close agreement with the in vivo effects of Ca^{2+}-channel blocking agents reported by Newcomb and Palma (1994). The Ca^{2+}-independent release of GABA and glutamate can be explained by reversal of the Na^+-dependent high-affinity uptake carriers (Erecinska, 1987; Nicholls, 1989), despite the presence of the uptake carrier blockers. In addition, we found that the basal release of GABA and glutamate, in the presence of SK&F 89976-A and L-*trans*-PDC, was not dependent on extracellular Ca^{2+}. Consistent with our results, other studies in different brain regions also showed that basal GABA release was practically Ca^{2+}-independent (Westerink and De Vries, 1989; Bourdelais and Kalivas, 1992).

4.2.2. Aspartate and taurine

Both basal and K^+-induced aspartate and taurine release were totally Ca^{2+}-independent in the presence of SK&F 89976-A and L-*trans*-PDC. This observation suggests that their release is not by vesicular mechanisms, but probably by carrier-mediated release mechanisms. However, in vivo microdialysis studies in other brain areas have shown Ca^{2+}-dependent aspartate and taurine release (Sved and Curtis, 1993; Paulsen and Fonnum, 1989). Nevertheless, it is not likely that the aspartate released is of vesicular origin, since accumulation of this amino acid in vesicles has not been observed (Nicholls, 1989). Although it has been reported that synaptic vesicles may be enriched in taurine (Kontro et al., 1980), it is not clear whether K^+-induced taurine release is Ca^{2+}-dependent (Oja and Kontro, 1987). A possible indirect Ca^{2+}-dependent aspartate release mechanism has been proposed by Nicholls and Attwell (1990), who suggested that glutamate released by depolarisation in a Ca^{2+}-dependent manner is rapidly taken up by its carriers. This could induce an extra depolarisation, resulting in heteroexchange of aspartate from the cytosolic pool through the carrier. This would explain why we could not find any Ca^{2+}-dependent aspartate release since the uptake of glutamate was blocked by L-*trans*-PDC.

In conclusion, blockade of GABA and glutamate uptake carriers clearly influences basal and depolarisation-evoked extracellular levels of amino acids in the rat hippocampus in vivo. This indicates highly active clearance of these transmitters by their carriers in this brain region, thereby limiting the postsynaptic actions of these transmitters after release.

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