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Effect of depolarization on striatal amino acid efflux in perinatal rats: an in vivo microdialysis study

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We used in vivo microdialysis to determine if infusion of depolarizing concentrations of potassium stimulated striatal excitatory amino acid (EAA) efflux in post-natal day (PND) 7 rats. Dialysis probes were perfused with 100 mM KCl for 60 min ($n=6$); EAA efflux was unaffected until 40–60 min after onset of the infusion, when a trend towards increased EAA efflux was observed (glutamate $284 \pm 56\%$ of baseline). In animals exposed to 8% oxygen ($n=7$) before a more prolonged (100 min) KCl infusion, again over the first 40 min of KCl there were no changes in EAA efflux; subsequently, glutamate, aspartate and taurine efflux increased (peak values $682 \pm 187\%$, $228 \pm 32\%$, and $1208 \pm 437\%$ of baseline). These data suggest that in PND 7 rats a substantial contribution to basal striatal EAA efflux may be derived from non-neurotransmitter pools.

We recently developed a method for *in vivo* striatal microdialysis in post-natal day (PND) 7 Sprague–Dawley rats [8], which we have used to measure striatal extracellular fluid (ECF) amino acid efflux in immature rodent brain [16]. The ECF content of neurotransmitter amino acids presumably reflects synaptic release, in addition to release from neuronal metabolic pools, and from glia; however, the relative contributions of these three pools is difficult to delineate [14, 17, 18]. In mature experimental animals, in vivo microdialysis studies have demonstrated that severe hypoglycemia [3] and cerebral ischemia [1, 2, 7, 11] markedly increase ECF accumulation of excitatory amino acids (EAA); these increases may be attributable to suppression of neuronal and glial re-uptake and/or increased release (from neurotransmitter and/or metabolic pools) [10, 15].

In PND 7 rats, using microdialysis, we found that insulin-induced hypoglycemia stimulated glutamate and taurine efflux in striatum, but to a much lesser degree than that reported in adult brain, while aspartate efflux was unaffected [16]. In preliminary studies of hypoxia-ischemia-induced alterations in striatal amino acid efflux, transient bursts of increased glutamate (and taurine) efflux [9], rather than the sustained marked elevations in glutamate and aspartate efflux reported in adult brain, were detected. These differences from patterns in adult brain stimulated us to further charac-

terize striatal amino acid efflux at this developmental stage.

A practical strategy for assessment of the impact of the ionic milieu on neurotransmitter efflux is to alter the ionic composition of the microdialysis perfusion medium. We previously demonstrated that in PND 7 rats, microdialysis probe perfusion with solutions containing 100 mM potassium chloride (KCl), to elicit depolarization, markedly stimulated striatal dopamine efflux within 20 minutes [8]. In adult brain, both in vitro, in synaptosome preparations, and in vivo, depolarizing concentrations of KCl (30–100 mM) stimulate efflux of neurotransmitter amino acids from pre-synaptic nerve terminals, and from glia [6, 14, 19]. In this study, we determined the effects of KCl-induced depolarization on striatal amino acid efflux at this developmental stage. Based on our observations that hypoxic exposure increased the magnitude of depolarization-evoked dopamine release [8], we also examined the impact of moderate hypoxia (8% oxygen) on striatal amino acid efflux.

Microdialysis probes were implanted into the right striatum of ether-anesthetized PND 7 Sprague–Dawley rats (landmarks referenced to bregma: AP -1 mm, L 2.5 mm, D 5 mm [7]). Animals recovered for 2 h in warmed plastic containers (surface temperature maintained at 35.5°C); brain temperature was not monitored. The microdialysis protocol was approved by the University of Michigan Committee on Use and Care of Animals. Only data from pups with histologically verified probe placement were evaluated (about 2/3 animals). Probes were

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perfused with filtered modified Ringer's solution (NaCl 147 mM, KCl 4 mM, CaCl$_2$ 3.4 mM, pH 7.2) at 1.5 μl/ min (CMA 100 micro-infusion pump Bioanalytical Systems). To induce depolarization, a solution containing 100 mM KCl, 3.4 mM CaCl$_2$ and 50 mM NaCl (to maintain isotonicity) was substituted. It must be noted that perfusion solutions were not oxygenated, and all samples collected from perfused striatum may, to some degree, have reflected local tissue hypoxia.

In preliminary experiments (data not shown), we were surprised to find that infusion of KCl (100 mM) for 20–30 min did not alter amino acid efflux. Two protocols were used to evaluate this response in greater detail. In the first group, 3 sequential 20 min baseline samples were collected in all pups ($n = 12$); then, probes were perfused with a solution containing 100 mM KCl and 3 more samples were obtained ($n = 6$); 3 additional samples were also collected in controls ($n = 6$). In the second group, af-

TABLE I
STRIATAL AMINO ACID EFFLUX IN PND 7 RATS: EFFECTS OF PERFUSION WITH 100 mM KCl

$n = 6$ animals/group. % Baseline: for each animal, the mean of the efflux values in the first 3 samples collected was calculated, and subsequent values were expressed as % of this mean. Group means were calculated subsequently. After insertion of a microdialysis probe into the right striatum of PND 7 rats (see text for details of method), and a 2 h recovery period, 6 sequential 20 min dialysate fractions were collected and analyzed for amino acid content. In controls, probes were continuously perfused with a modified Ringer's solution. In the experimental group after collection of the third fraction, perfusion with a solution containing 100 mM KCl, 3.4 mMCaCl$_2$, and 50 mM NaCl was initiated, and fractions 4–6 were collected.

Amino acid	Group	Fraction no. (% baseline)		
		4	5	6
Glutamate	KCl	75 ± 12	96 ± 10	284 ± 56
	Control	91 ± 20	70 ± 13	161 ± 62
Aspartate	KCl	61 ± 19	63 ± 20	140 ± 60
	Control	71 ± 13	66 ± 28	94 ± 24
Serine	KCl	89 ± 2	101 ± 14	107 ± 14
	Control	93 ± 8	80 ± 18	83 ± 26
Taurine	KCl	95 ± 12	105 ± 9	264 ± 78
	Control	101 ± 11	105 ± 8	340 ± 95
GABA	KCl	107 ± 14	131 ± 28	351 ± 122
	Control	84 ± 17	112 ± 22	84 ± 17
Glycine	KCl	72 ± 12	86 ± 9	115 ± 20
	Control	107 ± 12	100 ± 10	159 ± 59

ter 2 baseline samples were collected, animals were exposed to 8% O$_2$ ($n = 7$) for 60 min before initiation of the KCl infusion. The KCl infusion was continued for 60 min in hypoxia and for an additional 40 min in room air; 10 sequential fractions were also collected from 3 untreated controls.

After a pre-column derivatization, samples were analyzed by HPLC [5, 16]. Samples (30 μl) were mixed with 22.5 μl of the derivatizing solution (27 mg o-pthaldialdehyde / 2 ml 100 mM borax, with 20 ml mercaptoethanol, pH 9.5) for 2 min before injection onto a C18 reverse phase column (5 μ ODS, Altex); the mobile phase was 0.1 M NaH$_2$PO$_4$, pH 6.5 with 35% methanol. A glassy carbon electrode connected to an electrochemical detector (Bioanalytical Systems) was used (sensitivity 20 nAmp, electrode potential 0.7 V vs Ag/AgCl). Glutamate, aspartate, glutamine, glycine, asparagine, taurine, serine, and alanine were consistently detected in dialysates; γ-aminobutyric acid (GABA) was detected intermittently. Values were quantified by measurement of peak heights, and comparison with peak heights of external standards.

Efflux values were expressed as % of baseline. Baseline values were averaged from the first three dialysate samples in each animal, in the first group of experiments, and from the first two samples collected in the second group. Descriptive statistics, analysis of variance (ANOVA) for repeated measures, and Mann–Whitney U-tests were performed using a microcomputer based statistical package (Statview).

Absolute values for amino acid efflux were in the same range as previously reported (1.5 – 2 pmol/min for glutamate and aspartate)[16]. In controls, amino acid efflux did not change over 3.5 h (Table I, Fig. 1). Initially, infusion of 100 mM KCl had no effect on amino acid efflux. Over the first 40 min of the infusion, in two sequential dialysate samples amino acid efflux remained stable; in the final sampling period, 40–60 min after initiation of the KCl infusion, mean efflux values for glutamate, taurine, and GABA were elevated to > 250% of baseline but there was considerable inter-animal variation (Table I; repeated measures ANOVA for each amino acid, values expressed as% baseline, P = n.s.).

With the second experimental protocol which included both hypoxic exposure and a longer (100 min) period of KCl infusion (Fig. 1), more consistent increases in glutamate, aspartate, and taurine release were detected. Exposure to 8% O$_2$ (Fig. 1, Fractions 2–4) did not alter glutamate, aspartate, or glutamine efflux, while taurine efflux rose 2- to 3-fold. As in the first group of experiments, in hypoxic animals, KCl infusion initially did not stimulate efflux (Fractions 5–6); however, again, from 40–60 min (Fraction 7), glutamate and aspartate efflux
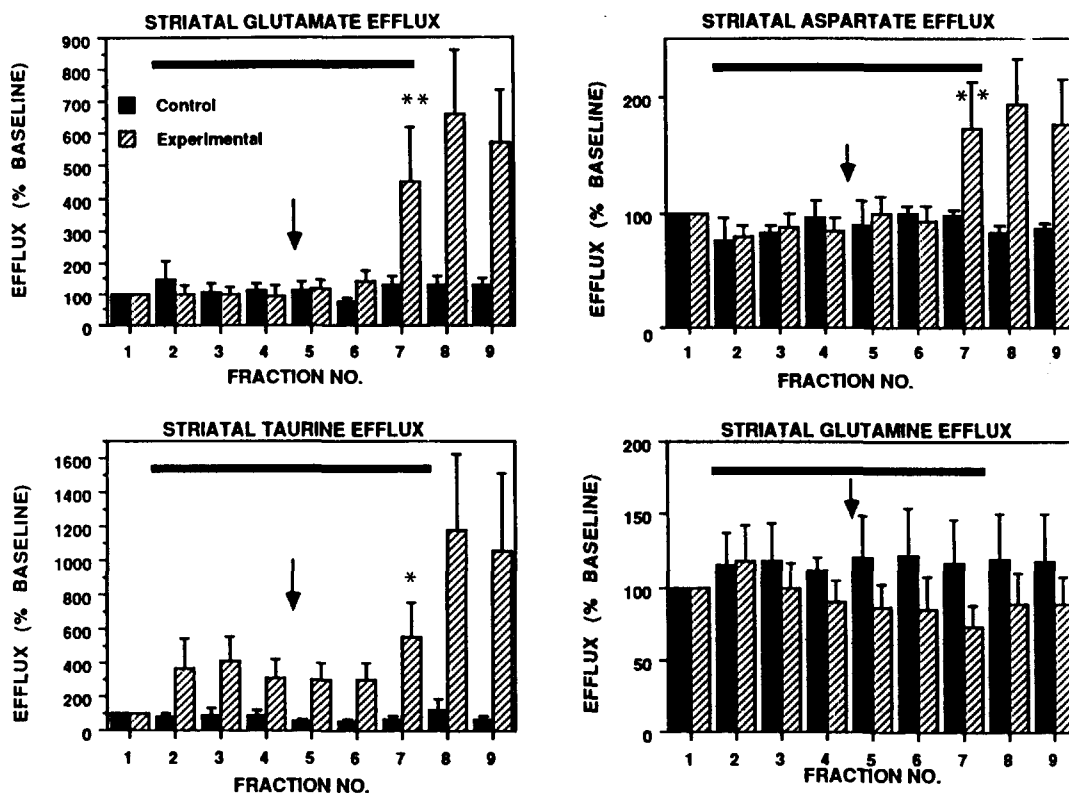
Fig. 1. Graphs demonstrating sequential striatal microdialysis efflux values, expressed as % of baseline, of glutamate, aspartate, taurine, and glutamine, in animals exposed to 8% oxygen and perfused with 100 mM KCl ('Experimental', $n=7$) and in controls ($n=3$). Microdialysis probes, perfused with modified Ringer's solution, were inserted into the right striatum of PND 7 rats (see text for experimental methods). For each animal, baseline values (Fraction 1) were averaged from the first two dialysis samples (20 min/sample). Solid horizontal bars indicate period of exposure to 8% oxygen (Fractions 2–7). Arrow indicates initiation of perfusion with 100 mM KCl containing solution, $**P=0.017$, $*P=0.053$, Mann–Whitney U-test comparing peak efflux values in experimental and control animals.

increased (to 448 ± 171 and $173\pm41\%$ of baseline, respectively). These increases were sustained over the next 40 min, while the KCl infusion was continued in room air. Taurine efflux increased concurrently. The magnitude and timing of maximal stimulation varied considerably; peak increases, for glutamate, aspartate and taurine were $682\pm187\%$, $228\pm32\%$, and $1208\pm437\%$ of baseline ($P=0.017$, 0.017, and 0.053, respectively, comparing peak values, expressed as % of baseline, in experimentals and controls with Mann–Whitney U-tests). In these samples GABA was not consistently detected, and efflux could not be analyzed. A trend towards declines in glutamine efflux was apparent (Fig. 1); depletion of glutamine, a glutamate precursor, could reflect stimulated glutamate efflux, or inhibition of glutamine synthetase. Glycine, serine, alanine, and asparagine efflux were unaffected by hypoxic exposure or KCl infusion.

An obvious question is whether depolarizing concentrations of KCl were attained in the first 40 min of the infusion; since, with the same methods, a >8-fold rise in dopamine efflux was observed within 20 min [8] it is likely that KCl concentrations in striatum were sufficient to elicit depolarization. Although the long sampling period

required for these studies may have obscured subtle or transient stimulation of EAA release, it is more likely that other factors accounted for the initial unresponsiveness to KCl.

The ECF content of EAA is derived from 3 sources: neuronal neurotransmitter pools, neuronal metabolic pools, and glia. At this developmental stage, the concentration of functionally responsive glutamatergic nerve terminals [4] and the amount of neurotransmitter released/neuron may be lower than in the mature brain. If the neurotransmitter pool yielded a relatively small contribution to EAA efflux as compared with metabolic pools, then even with stimulation of neurotransmitter release, little change in total ECF content might be detected. The relatively larger ECF volume in immature brain might contribute an important dilutional effect. Furthermore, depolarization-evoked EAA release from glia may be considerably lower at this developmental stage than in mature brain [14]. These factors, in combination, provide the most likely explanation for our results.

Of note, consistent with results in adult brain [14], sustained depolarization did eventually elicit selective sti-

mulation of glutamate, aspartate, taurine, and perhaps, GABA release. Relatively low reuptake rates [4] may have contributed to the gradual accumulation of these amino acids. The greater magnitude of KCl-evoked EAA release after hypoxia may, in part, reflect depolarization of a larger number of neurons with more prolonged exposure. However, comparison of EAA efflux at corresponding time intervals of KCl infusion indicated that values were more consistently elevated in hypoxic animals. Previously, we found that hypoxic exposure markedly increased the magnitude of KCl-stimulated dopamine release (to > 25-fold)[6]. Contributing mechanisms in both instances could include increased size of the releasable pool (as a result of decreased impulse flow during hypoxia), increased synthesis or intracellular translocation to releasable pools, or hypoxia-induced suppression of re-uptake [15].

Prominent increases in taurine efflux occurred concurrently with stimulation of glutamate efflux. In the immature striatum, both hypoglycemia and hypoxia-ischemia stimulated striatal taurine efflux to a similar extent [7, 13]. It is uncertain if the increased taurine efflux represents an adaptive response to increased glutamate release [12, 13], or an independently regulated event.

Our results suggest that at this developmental stage, a substantial fraction of basal striatal ECF EAA content may be derived from non-neurotransmitter pools. If the neurotransmitter pool contributes less to EAA efflux, then detection of depolarization-evoked EAA release with in vivo microdialysis may be considerably more difficult than in adult brain. These observations should be considered in analyzing data from microdialysis studies of amino acid efflux in immature rodent brain.

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