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BRAIN RESEARCH

Research Report

# Impaired formalin-evoked changes of spinal amino acid levels in diabetic rats

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STZ, streptozotocin
GABA, gamma amino butyric acid
CGRP, calcitonin gene related peptide
CSF, cerebrospinal fluid
ACSF, artificial cerebrospinal fluid

ABSTRACT

To investigate mechanisms by which diabetes alters sensory processing, we measured levels of amino acid neurotransmitters in spinal dialysates from awake, unrestrained control and diabetic rats under resting conditions and following hind paw formalin injection. Under resting conditions, glutamate concentrations in spinal dialysates were significantly ($P<0.05$) decreased in diabetic rats compared to those of control rats whereas aspartate, taurine, glycine and citrulline remained unchanged and GABA was significantly ($P<0.05$) increased. Noxious stimulation of the hind paw by subcutaneous injection of 0.5% formalin into the dorsum caused a defined flinching behavior in the afflicted paw, and the amount of flinching was significantly ($P<0.05$) greater in diabetic rats than in controls. Paw formalin injection significantly ($P<0.05$) increased dialysate levels of glutamate, aspartate, taurine, glycine and citrulline by 3- to 4-fold above basal in both control and diabetic rats. The concentration of glutamate in dialysate samples collected immediately after paw formalin injection remained significantly ($P<0.05$) lower in diabetic rats compared to those in controls. Formalin injection did not alter dialysate GABA concentrations in control rats, whereas in diabetic rats there was an increase of 151±15% above basal levels. These findings indicate that the selective depression of basal and stimulus-evoked glutamate levels in the spinal cord of diabetic rats occurs in parallel with elevated spinal GABA levels. Because increased pain-associated behavior is accompanied by an attenuated spinal glutamate spike following paw formalin injection, hyperalgesia in diabetic rats does not appear to be secondary to enhanced glutamatergic input to the spinal cord.



## 1. Introduction

Rats exposed to short-term diabetes show behavioral characteristics of enhanced sensory function such as tactile allodynia and mechanical, thermal and chemical hyperalgesia that have been used as models of painful diabetic neuropathy (Calcutt, 2000). Proposed pathophysiologic mechanisms have focused on increased sensitivity or activity of primary afferents, and accumulating electrophysiologic evidence supports this (Ahlgren et al., 1992; Craner et al., 2002; Khan et al., 2002). However, the contribution of these phenomena to behavioral allodynia and hyperalgesia is less clear, and they occur in the context of a developing neurochemical and structural phenotype that is more reflective of degeneration

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and sensory loss. For example, both synthesis and axonal transport of substance P and calcitonin gene related peptide (CGRP) are reduced in diabetic rats (Diemel et al., 1992; Robinson et al., 1987), and this contributes to diminished release of these neuropeptides from both peripheral and central terminals of primary afferents following electrical or chemical stimulation (Calcutt et al., 1998, 2000a; Garrett et al., 1997). Such findings suggest that behavioral hyperalgesia in diabetic animals unlikely results from enhanced spinal neuropeptide release.

While diabetes is associated with decreased peptidergic input to the spinal cord, little is known of the effects on either the excitatory amino acid neurotransmitters released from central terminals of primary afferents or the inhibitory amino acids that modulate spinal nociceptive processing. Pharmacologic studies have caused a number of authors to speculate that increased spinal glutamatergic input could contribute to hyperalgesia in diabetic rats (Gupta et al., 2003; Malcangio and Tomlinson, 1998; Zhang et al., 2002), but no direct measurements have been reported.

We have previously shown that levels of the excitatory neurotransmitter glutamate increase in spinal cerebrospinal fluid (CSF) of control rats immediately following injection of formalin into the hind paw and that this behavior is concurrent with flinching of the afflicted paw (Malmberg and Yaksh, 1995; Marsala et al., 1995). Diabetic rats show an increased frequency of flinching following paw formalin injection that is indicative of hyperalgesia in this model (Courteix et al., 1993; Malmberg et al., 1993). The formalin test is therefore a useful model in which to establish whether hyperalgesic behavior in diabetic animals is associated with elevated or protracted glutamatergic input to the spinal cord. To test this hypothesis, we measured amino acids levels in spinal CSF of conscious, unrestrained control and diabetic rats using in vivo microdialysis techniques both before and after paw formalin injection.

## 2. Results

### 2.1. *Basal and formalin-evoked levels of amino acids in spinal dialysates and concurrent behavioral responses*

Streptozotocin-injected rats were hyperglycemic (32.0±1.8 vs. 5.2±0.3 mmol/l) and lighter (181±8 vs. 240±7 g) than age-matched controls (both $P<0.01$, unpaired $t$ test) at the conclusion of the study. Flinching behavior of the paw that received formalin injection was measured concurrent with spinal microdialysis. Control rats exhibited a bi-phasic flinching response with the active phases separated by a quiescent period of inactivity (Fig. 1). In diabetic rats, flinching was increased within minutes of the injection of formalin, and this increase was maintained throughout all three phases of the monitoring period (all $P<0.05$ vs. controls, unpaired $t$ test).

Basal concentrations of the excitatory amino acid glutamate in spinal dialysates were significantly ($P<0.05$, unpaired $t$ test) lower in diabetic rats than in controls (Table 1 and Fig. 2), whereas there was no difference in basal levels of aspartate, taurine, glycine or citrulline between control and diabetic rats (Table 1).

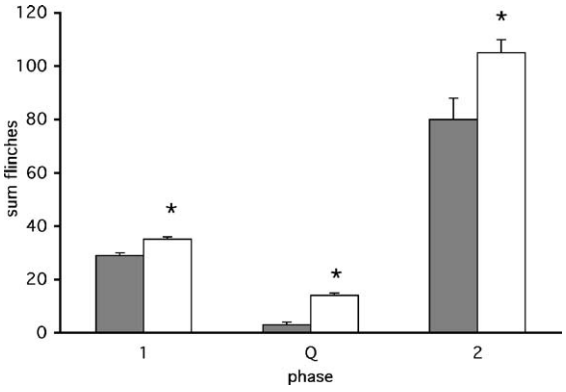


**Fig. 1 – Formalin-evoked paw flinching, presented as sum flinches counted during minutes 0–1,1–2, 2–3, 3–4 and 4–5 (phase 1), during minutes 5–6, 10–11 and 15–16 (phase Q) and during minutes 20–21, 25–26, 30–31, 35–36, 40–41, 45–46, 50–51, 55–56 and 60–61 (phase 2) of the formalin test in control (filled bars) and diabetic (open bars) rats. Data are mean±SEM of $N=7$–8 rats per group. *$P<0.05$ vs. control by unpaired $t$ test.**

Injection of 0.5% formalin into the hind paw of control rats evoked a significant ($P<0.01$, paired $t$ test) increase in glutamate above basal levels that was most marked in the first fraction following paw stimulation (Table 1 and Fig. 2). Transient increases of aspartate, taurine, glycine and citrulline were also noted in the fraction immediately following formalin injection (Table 1). Diabetic rats also exhibited a marked increase in glutamate levels above baseline following paw formalin injection. Absolute levels were significantly ($P<0.05$, unpaired $t$ test) lower in the fractions collected during the initial 10 min after formalin compared to those of control rats (Fig. 1), although the change relative to basal levels of the same animals was of a similar magnitude to that seen in control rats (Table 1). Aspartate, taurine, glycine and citrulline levels also increased in CSF dialysates from diabetic rats in the first 5 min after paw formalin injection and were similar to controls in both absolute levels and relative increase above basal levels (Table 1).

### 2.2. *Basal and formalin-evoked GABA levels in spinal dialysates*

GABA was measured in a separate cohort of control and diabetic rats. Streptozotocin-injected rats were hyperglycemic (30.9±2.3 vs. 6.1±0.4 mmol/l) and had reduced body weight (187±10 vs. 240±5 g) compared to age-matched controls (both $P<0.01$ by unpaired $t$ test). Glucose levels in CSF collected at the time of dialysis fiber implantation were also significantly ($P<0.01$) higher in diabetic rats (7.7±0.4 mmol/l) compared to controls (2.4±0.1 mmol/l). Basal GABA concentrations in spinal dialysate were higher ($P<0.05$ by unpaired $t$ test) in the diabetic rats compared to those of control rats (Fig. 3). Injection of formalin into the hind paw of control rats did not alter GABA concentrations in spinal dialysate collected in 10-minute fractions over the subsequent 60 min when compared to basal levels (Fig. 3). In contrast, diabetic rats exhibited a significant (151±15% of basal: $P<0.01$ by paired $t$ test) increase