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Research report

Determination of striatal extracellular  $\gamma$ -aminobutyric acid in non-hibernating and hibernating Arctic ground squirrels using quantitative microdialysis

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## Research report

Determination of striatal extracellular  $\gamma$ -aminobutyric acid in non-hibernating and hibernating Arctic ground squirrels using quantitative microdialysisP.G. Osborne<sup>1</sup>, Y. Hu, D.N. Covey, B.M. Barnes, Z. Katz, K.L. Drew<sup>\*</sup>*Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775-7000, USA*

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## Abstract

This study determined extracellular concentrations of  $\gamma$ -aminobutyric acid ( $[GABA]_{ecf}$ ) in striatum of non-hibernating and hibernating arctic ground squirrels to test the hypothesis that an increase in  $[GABA]_{ecf}$  was associated with profound CNS depression during hibernation. Quantitative microdialysis procedures were employed to circumvent the effects of low temperature on the relative recovery of the analyte across the dialysis membrane and yielded for the first time quantitative in vivo estimates of  $[GABA]_{ecf}$  in any brain region or any species. Laboratory housed, wild caught Arctic ground squirrels (*Spermophilus parryii*) were implanted intraperitoneally with radio transmitters that enabled the telemetric monitoring of activity and core body temperature ( $T_b$ ) and bilaterally implanted with cranial guide tubes that enabled the implantation of microdialysis probes into the striatum. Striatal  $[GABA]_{ecf}$  was determined in unrestrained, non-hibernating ground squirrels ( $T_b$  range 34.7–38.9°C) and hibernating ground squirrels ( $T_b$  range 2.9–3.9°C) using extrapolation to zero flow and very slow flow microdialysis techniques. The results show that  $[GABA]_{ecf}$  in non-hibernating squirrels was 73 nM and this level was decreased by approximately 50% during hibernation thereby suggesting that an increase in  $[GABA]_{ecf}$  does not play a major role in CNS depression during hibernation. The reduction of  $[GABA]_{ecf}$  parallels a decrease in plasma and CSF [glucose] and may be related to a decrease in GABA synthesis or reduced voltage dependent release. This paper demonstrates that measurement of extracellular concentrations of neurotransmitters in animals with vastly different body temperatures is possible using microdialysis techniques of extrapolation to zero flow or very slow flow rates that enable 100% recovery. Such quantitative techniques may prove valuable in the study of the neurochemistry of the cerebral mechanisms of hibernation and tolerance to cerebral ischemia exhibited by hibernating animals. © 1999 Published by Elsevier Science B.V. All rights reserved.

**Keywords:** Zero flow; Cold adaptation; Cerebral ischemia; GABA; Torpor

## 1. Introduction

Broadly, hibernation is a response to cold/freezing ambient temperatures and food shortage. It is composed of three distinct states; a brief cooling period, a long hibernation period and a brief re-warming period. Relative to non-hibernating mammals, hibernating mammals display enormous reductions of body temperature ( $T_b$ ) [1,4], metabolic rate [15], heart rate, cerebral blood flow [4], leukocytes [5], and platelets; and increased clotting time [16], isoelectric EEG and the absence of cerebral action potentials at  $T_b$  below 14°C [12]. The mechanisms by which these changes are initiated, maintained and reversed

are unknown. Although it is speculated that CNS control is probable, regional control has not yet been demonstrated.

GABA is the principal inhibitory neurotransmitter in the CNS, the over activation of which results in sedation and death, the inhibition of which results in behavioral activation, seizures and death [10]. Early studies proposed that cerebral excitability during hibernation may be attenuated or regulated by elevation of cerebral GABA [11]. These studies were limited to whole tissue determinations and yielded inconsistent results [9,11]. Since the inhibitory activity of GABA is dependent upon receptor activation and membrane hyperpolarization [10] the measurement of extracellular concentrations of ( $[GABA]_{ecf}$ ) will provide a more informative estimate of the functional role of GABA in hibernation.

The purpose of the present study was to measure  $[GABA]_{ecf}$  in hibernating and non-hibernating squirrel striatum to determine if the global cerebral depression

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observed during hibernation is mediated by an increase in  $[GABA]_{\text{ecf}}$ . We studied the striatum because (a) it is a large brain structure well suited to study in a species with no stereotaxic atlas and should be representative of the responses of other brain regions to the global neuronal depression during hibernation; (b) in rats, as probably in squirrels, it has dense GABAergic innervation and mechanisms of GABAergic efflux are well studied; and (c) because the striatum plays a critical role in the initiation of locomotor activity which is depressed during hibernation. To date, no studies have determined the  $[GABA]_{\text{ecf}}$  in any mammalian brain structure.

We used the microdialysis technique to sample the extracellular space [18]. To circumvent the effects of temperature on the relative recovery of GABA across the microdialysis membrane we utilized the in vivo quantitative technique of "extrapolation to zero flow" (EZF) [8,14] for determination of striatal  $[GABA]_{\text{ecf}}$  as it was most suited to conditions of greatly reduced cerebral blood flow [4] and metabolic rate [15]. As part of the EZF technique we used very slow perfusion speeds, which enabled 100% recovery across the dialyzing membrane [14]. We also measured systemic and cerebrospinal fluid glucose to determine if changes in striatal  $[GABA]_{\text{ecf}}$  paralleled those of GABA's principal substrate, glucose. In non-hibernating squirrels, striatal  $[GABA]_{\text{ecf}}$  was 73 nM and this was decreased by approximately 50% during hibernation. Plasma and CSF [glucose] were also found to decrease during hibernation.

## 2. Materials and methods

### 2.1. Animals

Arctic ground squirrels (*Spermophilus parryii*) were captured in the northern foothills of the Brooks Range in Alaska (66°38'N, 149°38'W) and transported to the Institute of Arctic Biology, University of Alaska Fairbanks. Animals were maintained on a diet of rodent chow, with daily supplements of carrots, apples, sunflower seeds and water ad lib. Animals were housed in the animal facility at 20°C and seasonally appropriate lighting down to 12:12 light/dark. Animals that did not hibernate were used as cold adapted, non-hibernating animals. A trapping permit (No. 96-50) was obtained from the State of Alaska Department of Fish and Game. All procedures were in accordance with the N.I.H. Guide for the Care and Use of Laboratory Animals and were approved by the UAF Institutional Animal Care and Use Committee. At the time of surgery body weight range was 719–993 g.

### 2.2. Surgery

Anesthesia was induced with methoxyflurane (Pitman-Moore, Mundelan, IL) and maintained with halothane (Halocarbon Lab, Riveredge, NJ) 1–3% mixed with 100%

medical grade O<sub>2</sub> at a flow rate of 1.5 l/min. During surgery, body temperature was maintained at 37°C with a servo-controlled fluid-filled heating pad (Omni medical equipment, Cincinnati, OH). Under sterile conditions, pre-calibrated, wax-coated temperature and activity telemetry transmitters (model VM-FH, Minimitter, Sunriver, OR) were implanted intraperitoneally. The squirrels were placed in a rat stereotaxic frame (Stoelting, Wooddale, IL) and guide cannulae for concentric microdialysis probes, 0.5 mm in diameter with a 4-mm dialyzing membrane at the tip (CMA 12, CMA, Acton, MA) were stereotaxically implanted into the right and left striatum. The skull was leveled at the points of ear bar zero (EBZ) + 10.0 mm and EBZ + 30.0 mm. The nose bar was then positioned at –20.0 mm relative to the value at level head and the guide cannulae were implanted using the AP coordinates from EBZ = +14.0 mm (the cranial suture that defines bregma is rarely visible in adult arctic ground squirrels),  $L = \pm 3.5$  mm from midline suture,  $D = -4.0$  mm from dura. Guide cannulae, containing an obturator, were secured with dental cement to stainless steel screws (BAS, West Lafayette, IN) implanted into the skull. A metal hook was also secured in the cement. After surgery squirrels were returned to their home cage and injected daily with chloramphenicol (35 mg/kg i.m.) for 10 days. Ibuprofen oral suspension (0.25 mg/ml, McNeil PPC, Washington, DC) was included in drinking water. A minimum of 10 days after surgery the squirrels were transferred to the hibernaculum, which had an ambient temperature of 2–4°C.

Microdialysis experiments were performed in the hibernaculum in cylindrical cages (approximately 0.6 m in diameter and 1 m in height), containing nest building material for hibernators or wood shavings for non-hibernators. Animals were moved to these cages 1–3 days after the initiation of the second bout of hibernation, or after at least 1 month of cold adaptation in squirrels that did not hibernate. Squirrels were secured to a counterbalance arm by attaching a flexible steel cable, containing perfusion lines, to both a plastic belt placed around the thorax and the metal hook embedded in the dental cement at the back of the implant. The dialysis cage was positioned directly over a telemetric receiver and  $T_b$  and body movements were monitored using telemetry (Dataquest III, Data Sciences, Minneapolis, MN, and Minimitter). On the first day of the experiment (day 1), the obturator was removed and a microdialysis probe was inserted into the left and right striatum.

Microdialysis probes were connected to a perfusion pump (CMA 102, CMA) via a liquid swivel (Instech Laboratories, Plymouth Meeting, PA) with low volume FEP tubing (CMA). Using a 1- or 0.1-ml gas-tight, glass, perfusion syringe (CMA) microdialysis probes were perfused with artificial cerebral spinal fluid (aCSF) containing 124 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl<sub>2</sub>, 0.85 mM MgCl<sub>2</sub>, 1.4 mM glucose, 24 mM NaHCO<sub>3</sub>, adjusted to pH = 7.4, Po<sub>2</sub> = 70–80 mmHg, Pco<sub>2</sub> = 30–40 mmHg (by

bubbling with 95% N<sub>2</sub>/5% CO<sub>2</sub>). Microdialysis probes were implanted at approximately 10:00 h and perfused at 0.6 μl/min for 5 h. Hourly samples were collected at 0.6 μl/min, then the flow rate was reduced to 0.05 or 0.1 μl/min overnight. One low flow sample was collected from each 9–12-h overnight period. This perfusion protocol was repeated for 3 days in non-hibernating squirrels and 5 days in hibernating squirrels. Only samples collected after [GABA]<sub>dia</sub> had stabilized were used for subsequent analysis. For these samples, stability was defined as [GABA]<sub>dia</sub> within 20% of the next sample. In vivo recovery at 0.6 μl/min was calculated by dividing [GABA]<sub>dia</sub> collected at 0.6 μl/min by [GABA]<sub>ecf</sub> estimated from the mean of the very low flow samples.

### 2.3. Extrapolation to zero flow

The concentration of GABA in microdialysis samples ( $C_{dia}$ ) collected at 0.05, 0.1 and 0.6 μl/min were fit as a function of flow rate according to the model developed by Jacobson et al. in 1985 and further verified by Parsons and Justice [14]. The model describes the relationship between flow rate, membrane area and mass transfer in a hollow cylinder with semipermeable walls (Eq. (1)), where  $C_{dia}$  is the concentration of GABA in the dialysate,  $C_{ecf}$  is the concentration of GABA in the extracellular fluid,  $F$  is the flow rate through the microdialysis probe in μl/min, and  $K$  is a constant that represents the product of the mass transfer coefficient and the active area of the dialysis membrane. Using this model, it is possible to extrapolate to a flow rate of zero and estimate  $C_{dia}$  at zero flow and hence [GABA]<sub>ecf</sub>.

$$C_{dia} = C_{ecf}(1 - e^{-K/F}). \quad (1)$$

Curves were fit with non-linear least squares regression, using the Levenberg–Marquardt algorithm as implemented in FUDGIT version 2.33. (Copyright 1993, Martin D. Lacasse).

### 2.4. Very slow flows

$C_{dia}$  and  $C_{ecf}$  equilibrate when the rate of diffusion of the analyte into the dialysate across the dialyzing membrane exceeds or equals the flow of aCSF inside the dialysis membrane. Preliminary in vitro studies in solutions of known concentration (data not shown) showed that better fits for non-linear curves were obtained if at least one flow rate was slow enough to lie on the 100% recovery plateau near the y-intercept. Concentrations of GABA in dialysis samples obtained at the two slowest flow rates were compared to estimates of [GABA]<sub>ecf</sub> obtained using the EZF technique.

### 2.5. HPLC determination of GABA

GABA was analyzed using a previously published method [3]. Briefly, dialysates were derivitized with OPA

and *t*-butyl thiol and separated on a nucleosil C-18 column (100 × 4 mm, 3 μm) with a guard column (10 × 4 mm) using a mobile phase of 50 mM sodium acetate, 1.0 mM EDTA (pH = 5.4) and 51% acetonitrile pumped at 0.7 ml/min. The derivitized GABA was detected using an ESA 5100A electrochemical detector with 5011 analytical cell poised at 450 mV (vs. Ag/AgCl) and recorded on a Spectra-Physics (4270) integrator. GABA was quantified by comparing peak height to an external standard. Using this technique, GABA eluted at approximately 3.75 min with a limit of detection of 0.05 pmol.

### 2.6. Verification of microdialysis probe position

After each experiment, animals were euthanized with an overdose of methoxyflurane. The brains were removed, frozen and sliced on a freezing microtome. Sections were mounted on slides and stained for nissle substance with thionin. Results were not included if the dialysis probe was not in the striatum.

### 2.7. Determination of plasma and CSF [glucose]

In a separate experimental series, cold-adapted, non-hibernating ground squirrels ( $n = 6$ ), ground squirrels hibernating for less than 24 h ( $n = 2$ ), squirrels hibernating for 80% of their previous hibernation bout length ( $n = 4$ ) and one squirrel that was euthermic for less than 12 h after arousing from hibernation, were lightly anesthetized with methoxyflurane. Rectal temperatures were measured with a thermocouple thermometer and blood was withdrawn via cardiac puncture for analysis of glucose. Additional anesthesia (2 mg/kg xylazine/ketamine) was then administered intracardially and CSF was withdrawn via cisterna magna puncture for analysis of glucose. Plasma glucose was analyzed at 37°C on a Corning 860 blood gas analyzer (Chiron Diagnostics, Norwood, NJ). CSF glucose was analyzed on a 2300 STAT glucose/lactate analyzer (Yellow springs Instrument, Yellow springs, OH).

### 2.8. Statistics

Data were analyzed by ANOVA unless otherwise stated. Time was treated as a within subjects variable when included in the analysis. *T*-tests were performed when comparisons included only two means. Simple regression analysis was used to assess changes over time as well as to compare results obtained using extrapolation to zero flow and very slow flow techniques. All data are expressed as means ± S.E.M. Differences were considered significant at  $p < 0.05$ , two tailed.

## 3. Results

$T_b$  over the course of the dialysis experiments of non-hibernating squirrels was 34.7–38.9°C and hibernating

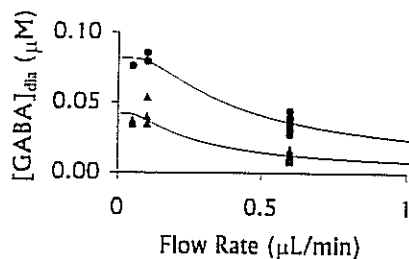


Fig. 1. Representative non-linear regression curves fit to data obtained from one non-hibernating (circles) and one hibernating (triangles) ground squirrel. Concentration of GABA in microdialysis samples ( $C_{dial}$ ) is plotted as a function of flow rate ( $F$ ) as modeled by Jacobson et al. [8] where  $C_{dial} = C_{ecf} (1 - e^{-K/F})$ .  $C_{ecf}$  is the concentration of GABA in the dialysate at a theoretical flow rate of zero and is therefore an estimate of concentration of GABA in the extracellular fluid.  $K$  is a constant that represents the product of the mass transfer coefficient and the active area of the dialysis membrane. Non-hibernating squirrel,  $C_{dial} = 0.0816 (1 - e^{-0.35/F})$ ; hibernating squirrel,  $C_{dial} = 0.0421 (1 - e^{-0.22/F})$ .

squirrels was 2.9–3.9°C. The ambient temperature of the hibernaculum was 2–4°C. Insertion of microdialysis probes did not induce an increase in  $T_b$  in the hibernating animals (data not shown).

### 3.1. Temporal profile of $[GABA]_{dial}$

Concentrations of GABA in dialysate samples collected at 0.6  $\mu\text{l}/\text{min}$  stabilized within 24 h of inserting the probes. In non-hibernating ground squirrels  $[GABA]_{dial}$  was not changed over sampling period of day 2 and day 3. In hibernating squirrels  $[GABA]_{dial}$  decreased over time (day 2–5) ( $p = 0.0051$ ). However, Newman–Keuls post-hoc analyses indicated only two points to be statistically different; the last sample collected on day 5 and the first sample collected on day 2 ( $p < 0.05$ ). Concentrations of GABA in dialysate samples collected at very slow flow rates (0.05 and 0.1  $\mu\text{l}/\text{min}$ ) typically stabilized by the second day, although some animals were stable by the first day. Within each group no difference in  $[GABA]_{dial}$  was found between flow rates (0.05 vs. 0.1  $\mu\text{l}/\text{min}$ ;  $p = 0.4177$ ); nor was there a significant correlation between sampling time and  $[GABA]_{dial}$  for each group ( $r^2 = 0.368$ ,  $p = 0.1485$  for non-hibernators,  $n = 7$ ;  $r^2 = 0.0846$ ,  $p = 0.209$  for hibernators,  $n = 19$ ).

### 3.2. Non-linear curve fitting (EZF)

Typical curves calculated from non-linear least squares regression of  $[GABA]_{dial}$  at flow rates of 0.6, 0.1 and 0.05  $\mu\text{l}/\text{min}$  for non-hibernating and hibernating squirrels are shown in Fig. 1. The  $y$ -intercept of each curve represents a steady state at which hypothetically the flow rate is zero and the concentration of GABA in the dialysate is equal to the concentration of GABA in the ECF [18]. Basal striatal  $[GABA]_{ecf}$  as determined from extrapolation to zero flow for non-hibernating squirrels ( $64.3 \pm 8.8$  nM;  $n = 3$  non-hibernating striata from three animals) tended to be higher

but was not significantly different from hibernating ground squirrels ( $42.5 \pm 5.4$  nM;  $n = 5$  hibernating striata from three animals) ( $t = 1.103$ ,  $p < 0.20$ ).  $K$  was not significantly different between the two groups ( $t = 1.720$ ,  $p < 0.1$ ).

### 3.3. Very slow flow

Comparison of  $[GABA]_{dial}$  collected at 0.05 and 0.1  $\mu\text{l}/\text{min}$  from hibernating and non-hibernating ground squirrels used in the EZF analysis showed a significant difference between the two groups ( $p = 0.0061$ , main effect of state). However, within each group no significant difference was found in the  $[GABA]_{dial}$  collected at 0.05 or 0.1  $\mu\text{l}/\text{min}$  ( $p = 0.42$ , main effect of flow rate). Nor was the interaction of state and flow rate (0.05 vs. 0.1  $\mu\text{l}/\text{min}$ ) statistically significant ( $p = 0.31$ ). Thus, in order to increase the sample size, samples from additional ground squirrels which had been collected at 0.1 but not 0.05  $\mu\text{l}/\text{min}$  were included in the subsequent analysis. For each animal, only a single mean value of all  $[GABA]_{dial}$  collected at either of the slow perfusion speeds was included in the analysis. Similarly, the corresponding mean  $[GABA]_{dial}$  from 0.6  $\mu\text{l}/\text{min}$  for these animals was analyzed. To facilitate comparisons between hibernating and non-hibernating ground squirrels, which were perfused for 5 and 3 days, respectively, only data through day 3 were used.  $[GABA]_{ecf}$  from perfusates collected at 0.1 or 0.05  $\mu\text{l}/\text{min}$  was  $73.1 \pm 11.6$  nM ( $n = 5$  animals) in non-hibernating animals and  $38.7 \pm 4.2$  nM, ( $n = 4$  animals) in hibernating animals,  $t = 2.51$ ,  $p < 0.05$ .  $[GABA]_{ecf}$  from perfusates collected at 0.6  $\mu\text{l}/\text{min}$  was  $60.8 \pm 14.2$  nM ( $n = 5$  animals) in non-hibernating animals and  $25.0 \pm 8.8$  nM, ( $n = 4$  animals) in hibernating animals,  $p = 0.08$  (Fig. 2). In vivo recovery of GABA at 0.6  $\mu\text{l}/\text{min}$  was  $83.8 \pm$

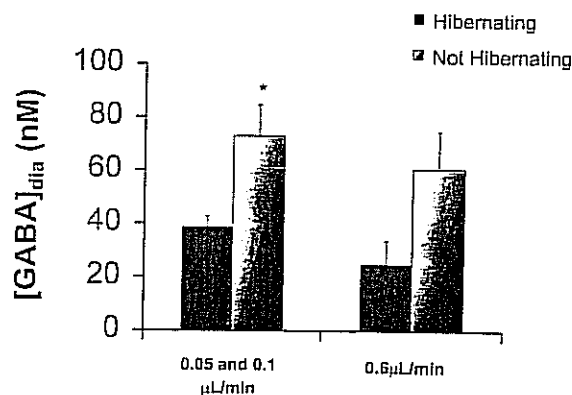


Fig. 2. Means of  $[GABA]_{dial}$  collected at slow flow rates (0.05 or 0.1  $\mu\text{l}/\text{min}$ ) and 0.6  $\mu\text{l}/\text{min}$  from non-hibernating ( $n = 5$ ; shaded histograms) and hibernating ( $n = 4$ ; filled histograms) ground squirrels. Only 1 point per animal per flow rate (mean of samples from left and right striatum on days 2–3). Concentrations of GABA in samples collected at the very low flow rates (0.05 and 0.1  $\mu\text{l}/\text{min}$ ) is an estimate of the concentration of GABA in the extracellular fluid ( $[GABA]_{ecf}$ ); \*  $p < 0.05$  between non-hibernating and hibernating squirrels at very low flow rates.

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14.6% in animals that were not hibernating ( $n = 5$  animals) and  $62.8 \pm 12.4\%$  in hibernating animals ( $n = 4$  animals,  $p = 0.33$ ) as estimated from striatal  $[GABA]_{ecf}$ .

#### 3.4. Hibernation-induced decreases in plasma and CSF glucose

In non-hibernating, cold adapted ground squirrels with mean  $T_b$  of  $36.6 \pm 0.3^\circ\text{C}$ , mean plasma concentration of glucose ( $[glucose]_p$ ) was  $10.0 \pm 0.9$  mM ( $n = 5$ ) (one sample was lost in assay).  $[Glucose]_p$  decreased to  $5.1 \pm 0.5$  mM ( $n = 4$ ) during prolonged hibernation (sampled at 80% of preceding bout length) when mean  $T_b$  was  $4.4 \pm 0.3^\circ\text{C}$  ( $p < 0.01$ ). Two animals sampled within 24 h of the onset of hibernation, having  $T_b$  of 4.7 and  $4.4^\circ\text{C}$  had  $[glucose]_p$  of 4.4 and 5.0 mM, respectively. Another animal sampled within 12 h of reaching near  $37^\circ\text{C}$  body temperature after a prolonged bout of hibernation ( $T_b = 35.1^\circ\text{C}$  at time of sampling) had  $[glucose]_p$  of 9.7 mM. These changes in  $[glucose]_p$  were also reflected in CSF glucose concentrations ( $[glucose]_{CSF}$ ). In non-hibernating, cold adapted ground squirrels  $[glucose]_{CSF}$  was  $6.1 \pm 0.5$  mM ( $n = 6$ ).  $[Glucose]_{CSF}$  decreased to  $3.2 \pm 0.5$  mM ( $n = 3$ ) during prolonged hibernation ( $t = 3.73$ ;  $p < 0.01$ ).

#### 4. Discussion

The data reported herein does not support the theory that the profound neuronal depression associated with hibernation is mediated in part by an increase in GABAergic neurotransmission during hibernation. In fact, in the non-hibernating ground squirrel striatal  $[GABA]_{ecf}$  is approximately 73 nM and this is decreased by 50% during hibernation. It is unlikely that the decrease in striatal  $[GABA]_{ecf}$  is related to the quiescent state of the animal. In non-hibernating animals injections of GABA antagonists or inhibitors of GABA synthesis into rat striatum elicits body and limb shakes and tremors or gnawing behavior, depending on the site of injection [3]. The decrease in striatal  $[GABA]_{ecf}$  during hibernation could involve reduced GABA release consequent of a lack of cerebral action potentials [11] or reduced synthesis. Glucose is a principal substrate for GABA synthesis [10]. Using microdialysis, the determination of brain ECF  $[glucose]$  on sequential days is currently not possible [13]. As such, plasma and CSF glucose was used as an indicator of ECF glucose. Plasma  $[glucose]$  (Ref. [6], this paper) and CSF  $[glucose]$  are decreased by 50% during the onset of hibernation. Similar decreases in plasma glucose during hibernation have also been reported for two other species, European ground squirrels (*Citellus citellus*) [2] and *Citellus lateralis* [17]. However, glucose does not decrease in all hibernating ground squirrels [5]. Thus, although the temporal parallels with the decrease in  $[GABA]_{ecf}$  measured during hiberna-

tion are evident in this species, it is not known if reduced CSF and plasma glucose levels contribute to the decrease of striatal  $[GABA]_{ecf}$ .

This is the first report of quantitative estimates of striatal  $[GABA]_{ecf}$  in vivo.  $[GABA]_{ecf}$  determined in non-hibernating ground squirrel striatum is more than 10 times greater than extracellular concentrations of dopamine determined in rat striatum using quantitative microdialysis [14]. From a technical prospective, the present results establish that under these experimental conditions the very slow perfusion speeds employed are adequate for obtaining 100% recovery across the dialysis membrane in hibernating animals. The use of very slow flow offers considerable advantage over the more time and sample consuming 'extrapolation to zero flow technique' and will be useful in future investigations of the neural regulation of hibernation.

In most non-hibernating, homeotherms hypothermia is associated with the uncoupling of energy dependent, cellular membrane functions from energy generating processes which results in impairment of ion homeostasis and ultimately membrane potential [7]. Thus, the observation that striatal  $[GABA]_{ecf}$  was not increased but significantly decreased during hibernation demonstrates that membrane functions associated with GABA homeostasis are conserved and that a non-GABAergic mechanism is involved in the generation of the controlled, cerebral depression that is associated with hibernation. The apparent temporal relationship between GABA and glucose warrants further investigation.

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