Liquid chromatographic determination of GABA in microdialysis samples

APPLICATION NOTE

Why GABA ?

GABA is known to be a major inhibitory neurotransmitter in the CNS and is thought to play an important role in various neurological disorders.

Why Microdialysis?

Whole tissue analysis of GABA does not distinguish between intra- to extracellular GABA compartments (this ratio is reported to be about 9000 (1,2). It is thus a rather crude index of release and uptake activities of GABA neurones.

Similary, in vitro techniques (tissue slices, enriched neuronal or glial fractions, synaptosomes) disrupt the integrity of brain nuclei and neuronal pathways, thus eliminating the effects of other coexisting or interacting neurotransmitters.

In addition, these techniques can provide only one data point each time per animal. Using Microdialysis, one can obtain the whole response curve including control levels from a single animal.

Unique features, like sampling from a conscious animal, correlation to behavioural or neurological states, simultaneous analysis of a drug and a neurotransmitter and calibration, are completely new and specific only for Microdialysis.

Why HPLC with Electrochemical Detection?

GABA can be analysed by different methods: GC-MS, radioenzymatic assay, gradient HPLC with fluorescence detection. Very often these methods are not enough sensitive to be efficient when combined with Microdialysis.

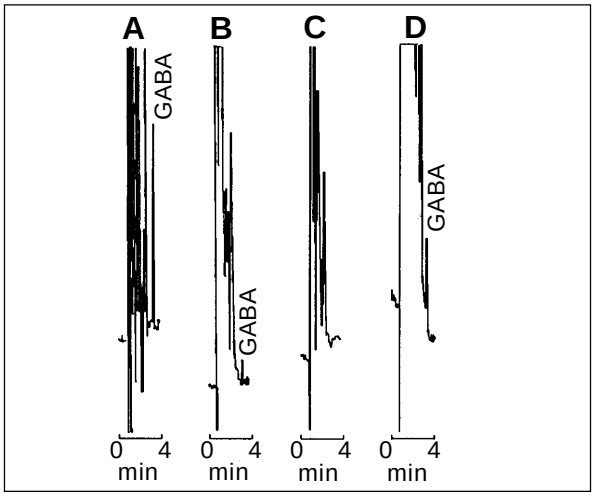
Common reagents for precolumn derivatization of GABA in liquid chromatography suffer either from instability of resulting derivative or that they are strong fluorophores themselves. Thus complicated pretreatment procedures are necessary, requiring extractions etc.

Recently, a new reagent based on o-phthalaldehyde/t-butylthiol reaction with primary amine groups was introduced (3). Resulting substituted isoindoles are stable (4) but significantly quench fluorescence. However, these derivates have strong electrochemical activities and can be oxidized on a carbon electrode giving high yields.

This was the basis for developing a method for determination of GABA by reverse-phase HPLC with electrochemical detection under isocratic conditions (5). With a 50 fmol detection limit, the method is the most sensitive procedure for GABA analysis today (6-10).

Is the GABA peak completely separated from other amino acids and interferences ?

Fig. 1. (A) GABA in mixture of 32 amino acids and related compounds, 0.5 pmol each. None of them interferes with the GABA peak.
(B) The limit of detection (at S/N ratio 2) is 50 fmol GABA.
(C) Blank, Ringer solution.
(D) Rat striatal microdialysis sample, CMA/10 Microdialysis Probe 3 mm membrane length, Ringer 2 µl/min, 5 min fraction.
Conditions:
C18 reverse-phase column, 3µm particle size, 0.15 M sodium acetate buffer, 1mM EDTA, pH 5.4, 50 % acetonitrile, flow rate 0.8 ml/min. Automated precolumn derivatization with o-phthalaldehyde/t-butylthiol reagent at 4°C.



Do extracellular GABA levels respond to pharmacological manipulations leading to the release and accumulation, or the reduction of extracellular GABA? (refs. 5,7,9,10)

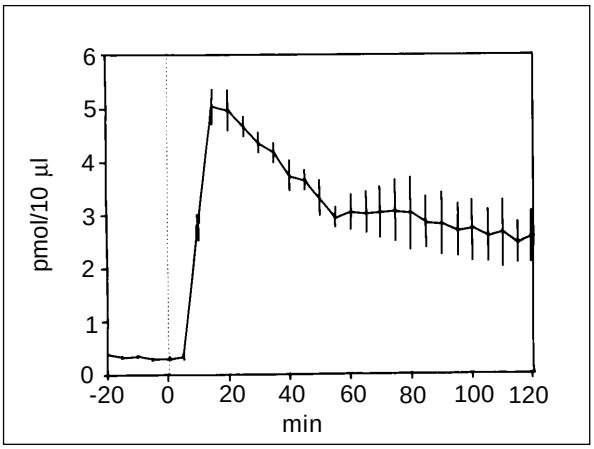


Fig. 2. Effect of the GABA uptake blocker, 0.5 mM nipecotic acid, infused via the probe on GABA levels in the rat striatum, mean ± SEM, n=4.

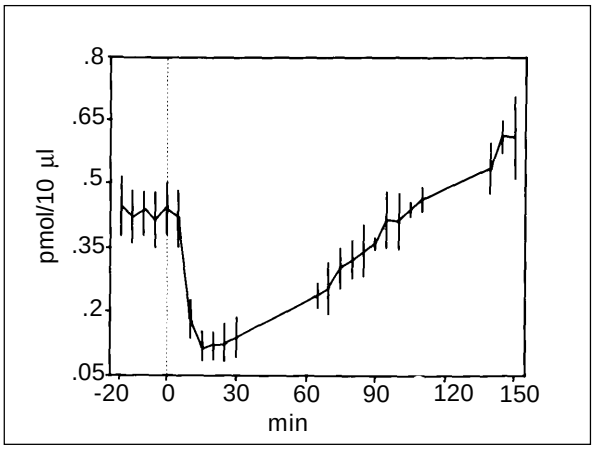
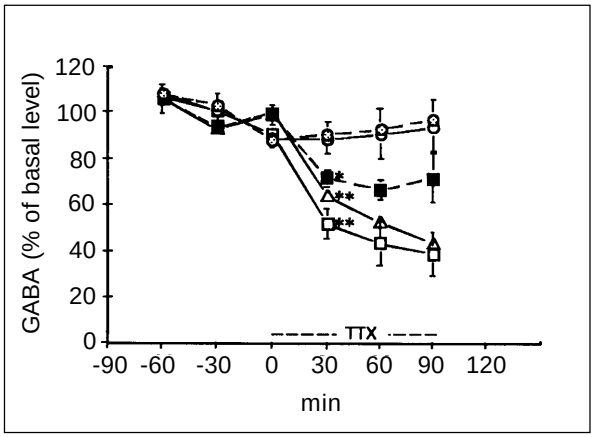


Fig. 3. Effect of GABA synthesis inhibitor, 3-mercapto-propionic acid, 100mg/kg i.p., on the extracellular levels of GABA in the rat striatum, mean ± SEM, n=4.

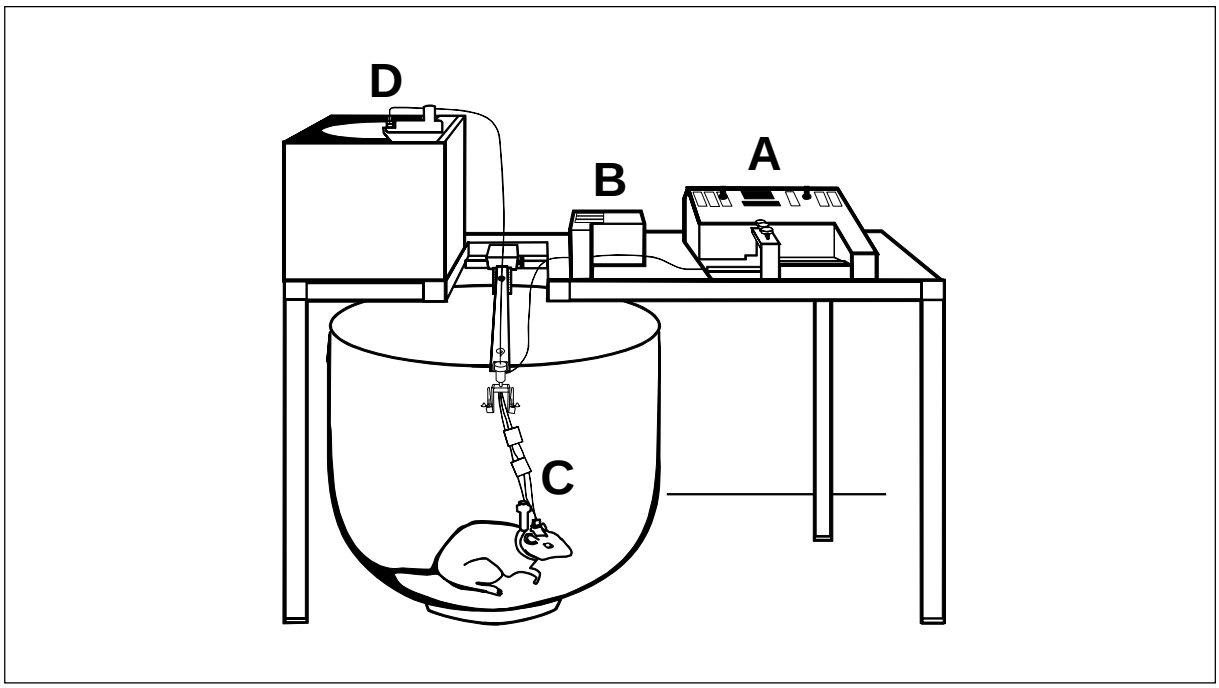
Is GABA overflow, sampled by Microdialysis, of neuronal origin?

Fig. 4. Effect of post-operative recovery and anaesthesia on the response of extracellular GABA to tetrodotoxin (TTX 1µM) in the rat striatum (8).
(○ ,n=4) 2.5 hr acute implantation, halothane anaesthesia
(■ ,n=8) 1 day after implantation, anaesthesia
(△ ,n=9) 1day after implantaion, conscious rats
(□ ,n=7) 4 days after implantation, conscious rats
(○ ,n=5) 1 day after implantation, control group
Data expressed as a percentage of the baseline levels from three samples preceding application of TTX (mean ±SEM).

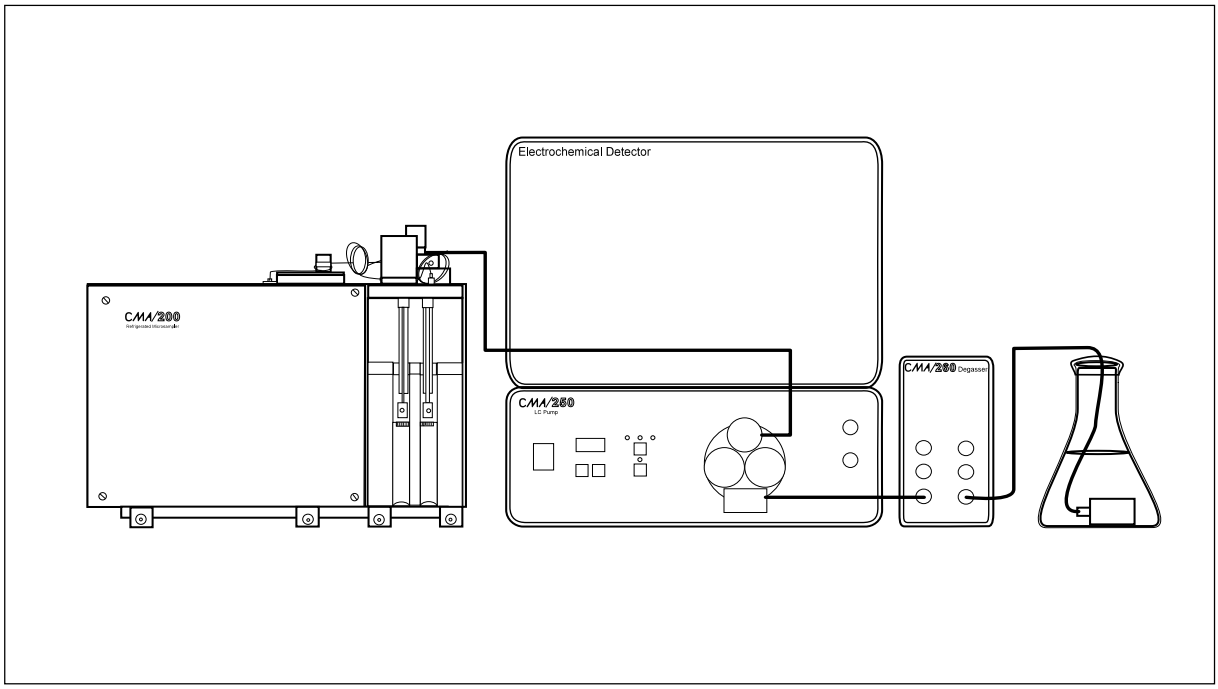


The voltage dependence of GABA overflow is charaterized by perfusion with the sodium channel blocker, tetrodotoxin (TTX), in different preparations. TTX does not inhibit GABA overflow in acutely implanted anaestethized rats (see also ref.6), but in conscious and chronically implanted (1-4 days) rats, the basal levels were reduced by 60 %. Similar patterns were observed by manipulating vesicular release (Ca^{2+} free perfusion, basal levels decreased by 52 %, ref. 9). These data suggest that at least 50 % of GABA overflow sampled by Microdialysis from freely moving rats is of neuronal origin.

What are the requirements for a successful experiment?



A complete Microdialysis System consisting of a **CMA/120 System for Freely Moving Animals** (table, cage and balancing arm) together with a **CMA/100 Microdialysis Pump (A)**, a **CMA/111 Syringe Selector (B)**, a **CMA/11 Microdialysis Probe (C)** and a **CMA/170 Refrigerated Fraction Collector (D)** or a **CMA/140 Microfraction Collector**.



The **CMA/200 Refrigerated Microsampler** has cooling down to 4°C and uses septa on the vials. This prevents samples from degradating and evaporating, as well as keeping the malodorous thiol-based reagent contained. The specially designed and patented dispensing system enables injection of almost the whole sample volume from a vial (only 0.5 µl excess volume is needed).

What is published about GABA analysis by HPLC/electrochemistry and GABA sampling by Microdialysis?

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3. Allison L.A., Mayer G.S., and Shorup R.E. (1984)o-Phthalaldehyde derivates of amino acids for high-speed liquid chromatography/ electrochemistry. Anal. Chem. 56, 1089-1096.

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11.Westerink B.H.C., and Devries J.B.(1989) On the origin of extracellular GABA collected by brain microdialysis and assayed by a simplified on-line method. Naunyn-Schmiedeberg´s Arch. Pharmacol. 339, 603-607.

If you require further details on Microdialysis procedures, HPLC analysis, instrumentation or bibliography, please do not hesitate to contact:

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