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In vitro microdialysis: a novel technique for stimulated neurotransmitter release measurements

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A novel microdialysis technique suitable for parallel measurements of neurotransmitter release and single unit recordings from brain slices is presented. The effects of 3,4-methylenedioxymethamphetamine (MDMA) on slices of dorsal raphe nucleus and frontal cortex in a perfusion chamber for electrophysiological measures were studied. MDMA caused measurable release of serotonin which, in the case of the dorsal raphe, was of similar duration as the period of reduced cell firing induced by MDMA. Tryptophan potentiated the action of MDMA. Possible additional applications of this technique are discussed.

Introduction

A powerful approach to the study of neuronal function and mechanisms of drug action is the combined measure of electrophysiological activity and biochemical indices of neuronal function (R th, 1987). Especially useful would be the direct measurement of neurotransmitter release concomitant with altered impulse flow. These combined studies have been successfully performed in whole animals using push-pull perfusion (Puizzillout et al., 1979) and in vivo voltammetry (Ewing et al., 1983; Kuhr et al, 1987), in combination with single unit recording. The brain slice preparation is quite attractive for this type of study because of th ability to control many more experimental variables than is possible in the whole animal (e.g. the concentrations of various ions can be altered and specific ion channel blockers can be added to determine ionic mechanisms of drug action; also, specific receptor agonists and antagonists can be added to the perfusion fluid to identify receptors mediating neurophysiological or pharmacological effects). In the present report we describe a method for making parallel electrophysiological and stimulated neurotransmitter release measurements from the surface of a brain slice.

The method we have developed is in vitro microdialysis, a modification of the well accepted technique of in vivo microdialysis (Ungerstedt, 1984). It employs a unique microdialysis assembly which can be lowered onto the surface of a brain slice in a rapidly perfused gas-fluid interface recording chamber. The collected dialysate is then directly injected into the chromatograph. An alternative method, the collection of superfusate from tissue chambers, is a classic technique (though it

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has not previously been coupled with electrophysiological recordings of single unit activity). The technique we have developed allows us to bypass the necessity of extracting the very small amount of neurotransmitter in the large volume a collected superfusate would represent. As an application of the methodology of in vitro microdialysis, experimental results are presented illustrating 3,4-methylenedioxymethamphetamine (MDMA)-induced 5-HT release from brain slices (dorsal raphe and cortex) in parallel with electrophysiological measurements (dorsal raphe). The data obtained from the dorsal raphe slice relate to release from the dendritic fields, while that from the cortex reflects release from axonal nerve terminal regions. Thus, this technique could potentially be of use in exploring differences in the regulation of release between these two regions. The electrophysiological data were obtained from a separate slice maintained in the same slice chamber while being perfused with the same artificial cerebrospinal fluid (ACSF). MDMA is an amphetamine analogue shown previously to release 5-HT from tissue in vitro (Nichols et al., 1982). Additional areas in which this technique could be of use are discussed.

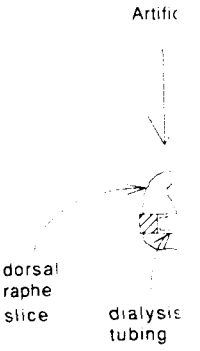
Materials and methods

Brain slice preparation and electrophysiological recording

Brain slices of dorsal raphe were obtained from male Sprague–Dawley rats (125–175 g) which had been anesthetized with chloral hydrate (400 mg/kg i.p.) and perfused with an ice-cold sucrose-enriched ACSF as described previously (Sprouse et al., 1989). The sucrose-enriched ACSF was of a standard composition (in mM): KCl 5.0, $CaCl_2$ 2.0, $MgSO_4$ 2.0, $NaHCO_3$ 28, NaH_2PO_4 1.25, D-glucose 10, except that NaCl, normally present at 126 mM, was replaced by a calculated equiosmolar concentration of sucrose (252 mM). The substitution of sucrose for NaCl in the ACSF for the preparatory and recovery phases of the experiment improved viability (Aghajanian and Rasmussen, 1989). Immediately following decapitation, the brain was removed rapidly, placed in a petri dish containing ice-cold sucrose-enriched ACSF and trimmed to expose the posterior midbrain. Coronal sections (500 μm) then were cut through the dorsal raphe using a vibrating-knife microtome (WPI, Vibroslice) and transferred to the stage of a gas-fluid interface brain slice chamber (Haas et al., 1979), where it was superfused at a flow rate of 1–1.5 ml/min. One hour from the time of decapitation, sucrose-enriched ACSF was replaced by standard ACSF containing NaCl. An additional period of 1 h was allowed prior to the experiment. Before initiating the release studies using dorsal raphe slices, 2.5 μM phenylephrine was added to the ACSF to induce the otherwise silent 5-HT neurons to fire (Vandermaelen and Aghajanian, 1983). Extracellular single unit recordings were performed using single barrel glass micropipettes (tip diameter 1–2 micrometer, 2–4 MΩ impedance at 60 Hz) filled with 2 M NaCl. Viability of the slice was determined by probing for active 5-HT neurons identified by their wide-duration action potentials (1–2 ms, positive–negative spikes), regular rhythm and slow firing rate (Vandermaelen and Aghajanian, 1983). Integrated firing rate was computed in 10-s samples. Agents were administered in the ACSF flowing over the slice by means of a stopcock arrangement.

Microdialysis

Microdialysis probes used for the neurotransmitter release studies were constructed using Cuprophan (Enka, West Germany) hollow fibers (300 μM i.d., 330 μM o.d.) housed in a section of 23-gauge stainless steel tubing. The fiber extended approximately 2.0–2.5 mm beyond the tip of the tubing exposing an active surface of 1.5–2.0 mm. An inlet section of 170 μm vitreous silica tubing (Scientific Glass Engineering) extended to the tip of the hollow fiber, while an outlet section was housed in the 23-G steel tubing with the tip above the other end of the dialysis fiber. The assembly was sealed within the section of the 23-G tubing using 5 min epoxy (Devco). Once the epoxy was completely cured, a gentle 90° bend was made near the tip of the assembly. The bend was made in several small increments using a pair of flat nose pliers in order to avoid a sharp right angle which can break the vitreous silica tubing.



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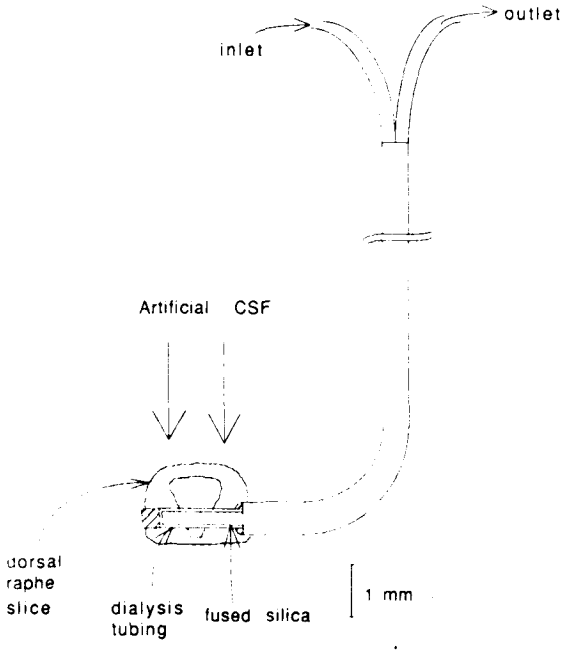

Fig. 1. Diagram of microdialysis assembly on a dorsal raphe brain slice. See Methods for details of assembly construction.

Perfusion buffer concentrations were (in mM): KCl 2.4, NaCl 120, $CaCl_2$ 1.2, $MgCl_2$ 1.2, NaH_2PO_4 0.9, Na_2HPO_4 1.4, ascorbic acid 0.3, pH 7.4. These concentrations correspond closely to the extracellular environment (Moghaddam and Bunney, 1989). Buffer was pumped through the assembly at a flow rate of 2.0 μl/min. The slice chamber resided in a Kopf stereotaxic apparatus, allowing the dialysis assembly to be lowered onto the slice surface using an electrode holder. A dissecting stereomicroscope was used to monitor the placement of the probe onto the slice, where it gently touched the surface. A diagram of a dialysis probe and representation of the experimental arrangement of the dialysis probe and the perfused brain slice is presented in Fig. 1. Collection periods were at 10-min intervals. The average "percentage recovery" (Ungerstedt, 1984) for 5-HT obtained with these probes in a static solution was 9.7 ± 0.7% (SEM) when perfused at 2.0 μl/min.

5-HT determination

5-HT in the dialysis perfusates was determined using reverse phase liquid chromatography with electrochemical detection. A laboratory designed and constructed pneumatic displacement fluid pump (Bradberry and Roth, in preparation) was used as a mobile phase pump. A battery powered potentiostat was used to apply a potential (+0.6 V versus Ag/AgCl reference) and convert the resulting current to a voltage for output to a stripchart recorder. Commercially available (Bioanalytical Systems) amperometric glassy carbon detector electrodes were used in conjunction with thin plastic spacers for maximum sensitivity (Mefford, 1987).

HPLC columns (10 cm × 2.1 mm i.d.) were packed in the laboratory with 3 micron C-18 material (Shandon). Mobile phase used for the experiments described herein was 0.05 M dibasic sodium phosphate, 350 mg/l sodium octanesulfonate, 0.1 mM disodium EDTA, 300 μl/l triethylamine, and 150 ml/l methanol (pH 5.6). The routine limit of detection for 5-HT obtained with this system was 2 fmol injected.

Results

Fig. 2 illustrates the effect of a 3 min exposure of 100 micromolar MDMA on the firing rate of a 5-HT dorsal raphe neuron. As can be seen, a long

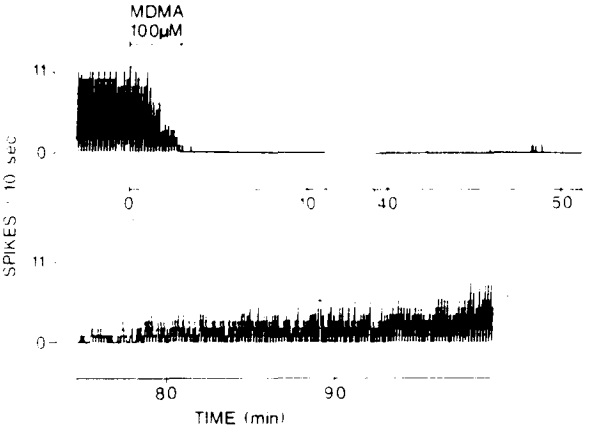

Fig. 2. Effects of 100 μM MDMA on the firing rate of a single dorsal raphe neuron. Application was for 3 min beginning at $t = 0$. By 100 min, firing rate had recovered to approx. 75% basal rate. Intervening sections not shown contained no significant activity. Highest level on firing rate histogram represents 11 spikes/10 s.

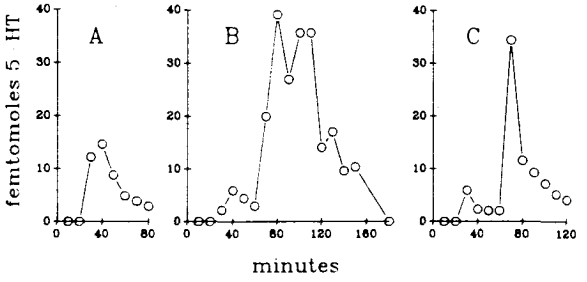

Fig. 3. Typical results from in vitro microdialysis release experiments. A: 5-HT release measurements from a slice of dorsal raphe. 100 μM MDMA was applied for 3 min starting at $t = 20$ min. B: the dorsal raphe slice in this experiment was treated with 500 μM Trp starting at $t = 20$ min through remainder of experiment. A 3 min application of 100 μM MDMA was given at $t = 60$ min. C: 5-HT release from a cortical slice exposed to Trp and MDMA as in panel B.

lasting period of complete inhibition results, with a gradual recovery to approximately 75% of basal firing rate at 100 min.

The parallel experiment (performed on a separate slice in the same chamber) in which a 3 min exposure of 100 μM MDMA is applied to a slice and endogenous 5-HT release is measured is presented in Fig. 3A. As can be seen, basal levels of 5-HT are below the limit of detection (< 2 fmol). MDMA application results in a long-lasting period of release of endogenous 5-HT. Superfusate collected during the period of maximal release for direct injection into the chromatograph contained levels below the limit of detection. This is due to dilution by the large volume of rapidly flowing superfusate, and illustrates the advantage of placing a probe directly on the surface of the brain slice. Comparing Figs. 2 and 3A, there appears to be a close temporal relationship between the period of 5-HT release and 5-HT neuronal inhibition.

Panel B of Fig. 3 illustrates an experiment in which a dorsal raphe brain slice is first pretreated with 500 μM tryptophan (Trp), the 5-HT precursor amino acid, prior to the 3 min application of 100 μM MDMA. The time of application of Trp is from $t = 20$ min on. In this case, Trp pretreatment raises basal 5-HT release to measurable levels. The MDMA-induced release from this Trp-pretreated slice is substantially increased compared to a slice not pretreated with Trp.

Panel C of Fig. 3 shows similar results from a slice of frontal cortex, a region containing terminal fields rather than cell bodies. The addition of Trp and MDMA are the same as in panel B.

Discussion

The results of the present study illustrate the applicability of in vitro microdialysis for making measurements of drug-induced release of endogenous 5-HT from both dorsal raphe and cortical brain slice preparations. MDMA has been shown previously to release labeled 5-HT from synaptosomes (Nichols et al., 1982), and brain slices containing 5-HT terminal fields (Schmidt et al., 1987). We have used our technique to temporally relate MDMA-induced release of 5-HT from slices of rat dorsal raphe with alterations in dorsal raphe 5-HT neuronal firing (Sprouse et al., 1989). The effect of Trp in the perfusion media to increase basal and MDMA-induced 5-HT release correlates with electrophysiological measurements demonstrating that Trp inhibits dorsal raphe firing and increases the potency of MDMA at inhibiting 5-HT neuronal firing (Sprouse et al., 1990). This parallel use of biochemical and electrophysiological techniques has allowed us to demonstrate a mechanism of action of MDMA inhibition of 5-HT neuronal firing whereby 5-HT neurons are inhibited by MDMA-released 5-HT acting at somatodendritic impulse regulating autoreceptors (Sprouse et al., 1989).

A possible alternative to placing a microdialysis assembly on the brain slice for measuring neurotransmitter release is to collect the superfusate for analysis. As indicated in Results, the concentration of 5-HT in the superfusate is much less than in the microdialysis perfusate, which would necessitate an extraction for subsequent 5-HT determination. Placing the microdialysis assembly directly onto the surface of the brain slice avoids this extra step.

Microvoltammetric methods have also been used for studying stimulated release of neurotransmitter release from brain slices (Kelly and Wightman, 1987; Bull et al., 1990). In these studies electrical stimulation in the presence of reuptake

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inhibitors elevated dopamine in striatal slices to measureable levels. Voltammetric methods are complimentary to microdialysis methods in that they offer increased time resolution, but are unable to match the slower microdialysis methods in sensitivity, which can detect basal concentrations as low as 1 nmol. In our case, a typical concentration limit of detection for dialysate injected into a chromatograph is 0.1 nmol, and our microdialysis probes, average roughly 10% recoveries.

The use of a dialysis probe on the surface of a brain slice offers additional advantages not specifically illustrated in this study. One intriguing possibility is the idea of selectively measuring release from different areas of a brain slice simultaneously. This would allow a distinction between release from different neuronal regions such as the cell body/dendritic region and nerve terminal regions. Also, this technique would be ideal for measuring release from a static (non-perfused) brain slice chamber, as is sometimes used for electrophysiological studies. In that preparation, removing samples without disturbing the recording electrode is not possible. A dialysis probe placed next to a recording electrode would provide a means of removing samples for analysis without disturbing the electrode. Additionally, dialysis probes can be used to administer drugs as well as collect samples (Ungerstedt 1984; Quan and Blatteis, 1989). Thus, a probe could serve as a means of delivering a test compound to the slice using a minimal volume of drug solution if the compound is expensive or is difficult to eject from a micropipet electrophoretically.

In summary, we have developed a novel method of measuring neurotransmitter release from the surface of a brain slice in an electrophysiological recording chamber, allowing correlative studies of the effects of compounds on neurotransmitter release and neuronal firing.

References

Aghajanian, G.K. and Rasmussen, K. (1988) Intracellular studies in the facial nucleus illustrating a simple new method for obtaining viable motoneurons in adult rat brain slices, Synapse, 3: 331–338.

Bull, D.R., Palij, P., Sheehan, M.J., Millar, J., Stamford, J.A., Kruk, Z.L. and Humphrey, P.P.A. (1990) Application of fast cyclic voltammetry to measurement of electrically evoked dopamine overflow from brain slices in vitro, J. Neurosci. Methods, 32: 37–44.

Ewing, A.G., Alloway, K.D., Curtis, S.D., Dayton, M.A., Wightman, R.M. and Rebec, G.V. (1983) Simultaneous electrochemical and unit recording measurements: characterization of the effects of D-amphetamine and ascorbic acid on neostriatal neurons, Brain Res., 261: 101–108.

Haas, H.L., Shaerer, B. and Vosmansky (1979) A simple perfusion chamber for the study of nervous tissue slices in vitro, J. Neurosci. Meth., 1: 323.

Kelly, R.S. and Wightman, R.M. (1987) Detection of dopamine overflow and diffusion with voltammetry in slices of rat brain, Brain Res., 423: 79–87.

Kuhr, W.G., Wightman, R.M. and Rebec, G.V. (1987) Dopaminergic neurons: simultaneous measurements of dopamine release and single-unit activity during stimulation of the medial forebrain bundle, Brain Res., 418: 122–128.

Mefford, I.N. (1987) Chromatographic approaches to signal and selectivity enhancement for determination of biogenic amines by liquid chromatography with amperometric detection, Life Sci., 41: 375–379.

Moghaddam, B. and Bunney, B.S. (1989) Sonic composition of microdialysis perfusing solution alters the pharmacological responsiveness and basal outflow of striatal dopamine, J. Neurochem., 53: 652–654.

Nichols, D.E., Lloyd, D.H., Hoffman, A.J., Nichols, M.B. and Yim, G.K. (1982) Effects of certain hallucinogenic amphetamine analogues on the release of [^{3}H]serotonin from rat brain synaptosomes, J. Med. Chem., 25: 530–535.

Puizzillout, J.J., Gaudin-Chazal, G., Daszuta, A., Seyfritz, N. and Ternaux, J.P. (1979) Release of endogenous serotonin from "encephale isolé" cats. II. Correlations with raphe neuronal activity and sleep and wakefulness, J. Physiol. (Paris), 75: 531–537.

Quan, N. and Blatteis, C.M. (1989) Microdialysis: A system for localized drug delivery into the brain, Brain Res. Bull. 22: 621–625.

Roth, R.H. (1987) Biochemical correlates of the electrophysiological activity of dopaminergic neurons: reflections on two decades of collaboration with electrophysiologists. In L.A. Chiodo and A.S. Freeman (Eds.), Neurophysiology of Dopaminergic Systems - Current Status and Clinical Perspectives, Lakeshore Publishing Co., Detroit, MI, pp. 187–203.

Schmidt C.J., Levin J.A. and Lovenberg W., 1987. In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain, Biochem. Pharmacol., 36: 747–755.

Sprouse J.S., Bradberry C.W., Roth R.H. and Aghajanian G. K., MDMA (3,4-methylenedioxymethamphetamine) inhibits the firing of dorsal raphe neurons in brain slices via release of serotonin, Eur. J. Pharmacol., 167: 375–383.

Sprouse, J.S., Bradberry, C.W., Roth, R.H. and Aghajanian,

G.K., MDMA (3,4-methylenedioxymethamphetamine)-induced release of serotonin and inhibition of dorsal raphe cell firing: potentiation by L-tryptophan, Eur. J. Pharmacol., 178: 313-320.

Ungerstedt U. (1984) In C.A. Marsden (Ed.), Measurement of neurotransmitter release in vivo, pp. 81–105. John Wiley & Sons, Chichester.

VanderMaelen, C.P. and Aghajanian, G.K. (1983) Electrophysiological and pharmacological characterization of serotonergic dorsal raphe neurons recorded extracellularly and intracellularly in rat brain slices, Brain Res., 289: 109–119.

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