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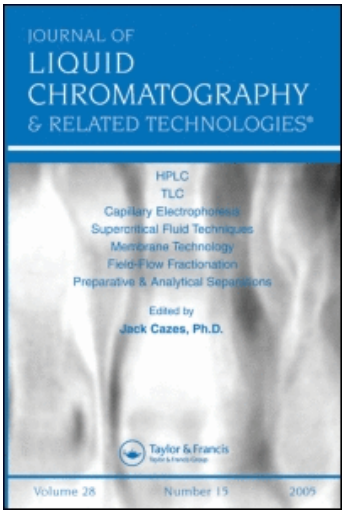

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
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Simultaneous Measurement of Monoamine, Amino Acid, and Drug Levels, Using High Performance Liquid Chromatography and Coulometric Array Technology: Application to *In Vivo* Microdialysis Perfusate Analysis

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To cite this Article Acworth, Ian N. , Yu, Jian , Ryan, Elizabeth , Gaiuepy, Kathleen Cox , Gamache, Paul , Hull, Keith and Maher, Timothy(1994) 'Simultaneous Measurement of Monoamine, Amino Acid, and Drug Levels, Using High Performance Liquid Chromatography and Coulometric Array Technology: Application to *In Vivo* Microdialysis Perfusate Analysis', Journal of Liquid Chromatography & Related Technologies, 17: 3, 685 — 705
To link to this Article: DOI: 10.1080/10826079408013169
URL: http://dx.doi.org/10.1080/10826079408013169

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JOURNAL OF LIQUID CHROMATOGRAPHY, 17(3), 685-705 (1994)

SIMULTANEOUS MEASUREMENT OF MONOAMINE, AMINO ACID, AND DRUG LEVELS, USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND COULOMETRIC ARRAY TECHNOLOGY: APPLICATION TO *IN VIVO* MICRODIALYSIS PERFUSATE ANALYSIS

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ABSTRACT

An automated HPLC coulometric array-ECD method is described for the simultaneous analysis of monoamines, their metabolites, derivatized amino acids, and pharmacological agents. This method has been used with in vivo microdialysis in urethane-anesthetized animals to examine extracellular fluid levels of endogenous and exogenous analytes after the peripheral administration of drugs. An aliquot of dialysate was initially analyzed for the monoamines, their metabolites and drugs by isocratic elution and detection on eight serial coulometric electrodes (0 to 490 mV; 70 mV increment). The remaining sample was then derivatized, pre-column, with OPA/ßME and, after column switching, was analyzed on a parallel isocratic system with detection on four electrodes (set at 250, 450, 550 and 650 mV respectively). Compounds were identified by their retention time and electrochemical profile across the arrays. This method had a limit of detection of 0.125 pg/µl for the monoamines and 0.75 pg/µl for amino acids (both with a signal to noise (S/N) ratio of 3:1). The detector response was linear over several orders of magnitude (0.25 to 20 pg/µl) for monoamines, their metabolites and the amino acids. The analysis was completed within 25 min.

A variety of drugs were also measured including: apomorphine (Apo), hydralazine (H), isoproterenol (Iso), methoxamine (Mx), morphine sulfate (M) and its metabolite morphine-3-glucuronide (M3G), and phenylephrine (Phe). The limit of detection for these compounds varied from 0.215 to 10.65 pg/µl (Phe

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and M3G respectively) with a S/N ratio of 3:1. The detector response was linear from 0.5-500 pg/µl and the linear regression correlation coefficient (r) varied from 0.9969 to 0.9998 (H and M3G respectively).

The peripheral administration of H (10 mg/kg i.v.) produced a 40% decrease in blood pressure (BP) and caused an almost immediate 220 fold increase in striatal dopamine (DA) levels. Levels of DOPAC and HVA decreased by 80-90% and those of the amino acids glutamate (GLU), aspartate (ASP), taurine (TAU) and gamma amino-butyric acid (GABA) increased 30-120 fold. Striatal levels of H reached a maximum of 9 pg/µl (405 pg/collection) 40 min after its administration. Nitroprusside (NPr) infusion (0.06-0.3 mg/min/kg i.v.) also decreased BP by 30%, increased striatal DA levels by 100 fold, and decreased levels of DOPAC and HVA by 40-50%. Although the amino acids were also affected, their levels began to increase only 140 min after the start of drug administration. NPr could not be detected using this method. In a separate experiment, hippocampal perfusate levels of M were found to reach a maximum of 12.6 pg/µl (567 pg/collection), 40min after its peripheral administration (10 mg/kg i.p.). Although M decreased hippocampal ECF levels of GABA and GLY, it appeared to have little effect on the other analytes measured.

This method not only makes it possible to study the interaction between different neurotransmitter pathways but also offers a more detailed inspection of the mechanism of drug action, a direct measure as to whether drugs pass through the blood-brain barrier (BBB) as well as direct acquisition of pharmacokinetic data.

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INTRODUCTION

The central efficacy of a drug may ultimately depend upon its level within the brain. Levels, in turn, are dependent upon several other factors including: dosage, route of administration, clearance (catabolism, metabolic conversion, excretion), tissue distribution and most importantly on its ability to pass the BBB. In the past, the central level of a drug was usually determined by examination of tissue homogenates with the inherent loss of compartmentalization, cellular distribution and the necessary use of large numbers of animals (for dose-response and temporal studies). Only recently have techniques become available with the potential for such studies to be done in vivo [for reviews see 1,2,3]. One such method, microdialysis, overcomes many of the above disadvantages and allows for continuous monitoring of the changes in a drug's level within discrete brain regions in a single animal [4-8]. Microdialysis has now become a routine sampling technique [9,10] which allows examination of the chemical microenvironment within the extracellular space of a variety of tissues including blood, adipose, liver and muscle. Most commonly used in nervous tissue, microdialysis is often coupled to HPLC analysis for examination of neurotransmitter release and metabolism in neurochemical, pharmacological, physiological or behavioral studies [see 11].

The central mechanism of drug action is often not exclusive to one particular neurotransmitter pathway but the drug may exert its effect via a dynamic interplay between various neurotransmitter systems which are involved at a primary, secondary or even tertiary level. The simultaneous measurement of the release and metabolism of different neurotransmitter systems should afford a better

understanding of a drug's action. Concurrent measurement of dialysate levels of the drug affecting neurotransmitter release is not often practical [12] but extremely desirable.

We report here an HPLC array-ECD method capable of measuring monoamines, their metabolites, amino acids and drug levels concurrently in microdialysis perfusates. We have used this technique to examine the effects of drug-induced hypotension on striatal neurotransmitter release and drug levels in perfusates obtained from the urethane anesthetized rat. This technique has also been used to examine the passage of morphine through the BBB and its effect on various neurotransmitter pathways. Finally, we have used this technique to resolve a variety of centrally acting drugs and metabolites.

METHODS

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Materials

HPLC grade ultra-pure water (Milli-Q, Millipore, Bedford, USA) >18 megaohm/cm^3 was further purified by passing through C18 solid phase extraction columns (Millipore Sep-Pak C18) to remove residual trace organics, and was used for preparation of all mobile phases and other solutions. o-Phthalaldehyde (OPA), beta-mercaptoethanol (ßME) and external standards were obtained from Sigma (St. Louis, MO, USA). Drugs utilized were either obtained from Sigma or RBI (Natick, MA, USA). All chemicals used in the HPLC analysis were of the highest grade available from either Sigma or Fluka (Buchs, Switzerland). Solvents were HPLC grade and were obtained from EM Science (Gibbstown, NJ, USA). Salts used in the artificial cerebro-spinal fluid (aCSF) perfusion medium were Microselect grade from Fluka.

Microdialysis

Male Sprague-Dawley rats (275-325 g) were anesthetized with urethane (2 g/kg i.p. with additional doses as necessary). Animals had both their carotid artery (connected to a Stathm pressure transducer and a Grass 79D polygraph for BP and heart-rate monitoring) and jugular vein (for drug administration) cannulated with heparinized PE50 tubing. After placement in a stereotaxic frame, the cranial skin was incised and the periosteum resected. A hole was drilled through the cranium above the right striatum

using a 1.8 mm trephine. A pre-calibrated 3 mm loop-design regenerated cellulose microdialysis probe (ESA, Inc., Bedford, MA, USA) was placed within the striatum using the following coordinates from Bregma (flat-skull): AP +0.7 mm; LR 2.7 mm; DV -7.5 mm from dura. Animals had their temperature monitored by rectal thermistor and were maintained at 37±0.5 °C using a heating pad. aCSF (based on the composition developed by Moghaddam and Bunney [13] but lacking ascorbic acid) was perfused through the probe at 1.5 µl/min using a Model 22 microperfusion pump (ESA, Inc.). Samples were collected every 20 min into 10 µl of 0.2 M perchloric acid (PCA) in order to minimize monoamine degradation.

For the experiments examining the passage of M through the BBB the above protocol was modified as follows: the animals were not vascularly cannulated; the probe was placed in the right hippocampus (from Bregma: AP -5.8 mm; LR 4.8 mm; DV -7.5 mm from dura); and M was administered intraperitoneally.

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Experimental Design

After placement of the probe within the specified brain region and after a 90-120 min period of injury-mediated monoamine release, samples were continuously collected and analyzed every 20 min. Following attainment of baseline (a steady-state situation defined as the period when levels of DA varied by less than 10% in at least three consecutive samples), the drug was administered. After a further 2-3 hr period, animals were euthanised by anesthetic overdose.

Drug Administration

Animals received either H (10 mg/kg bolus i.v., in physiological saline) or NPr (i.v. infusion; 0.06-0.3 mg/min/kg in saline, to decrease BP by 30%) or M (10 mg/kg i.p. in saline).

Probe Pre-calibration

The in vitro recovery of each probe was determined by perfusion of the probe with aCSF while placing the probe in a mixture of either external standards (1-3 µM) or drugs (1 mg/ml) dissolved in

acidified (0.1 M PCA) aCSF. After a period of 30 min (to achieve steady-state), 2-3 samples were collected every 20 min, at a flow rate of 1.5 µl/min and at a temperature of 24 °C. External standards were analyzed directly (drugs were analyzed after further dilution) as described below. The average probe recovery (with minimal inter-probe variability) was 30% for the monoamines, 40% for the amino acids and typically 15-18% for the various drugs studied. The same probe was able to be used in more than ten animals before decreased recovery compromised the analytical sensitivity. The in vitro recovery was only used to measure individual probe performance and inter-probe variability and not used to estimate extracellular fluid concentrations (this would be misleading as the diffusion kinetics in vivo are known to differ from those in vitro [14,15]).

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HPLC Coulometric Electrode Array Analysis

Samples were analyzed with a Coulochem Electrode Array System (CEAS, Model 5500, ESA Inc.) in dual isocratic mode consisting of a Model 465 autoinjector, two Model 420 dual piston pumps, a ten port switching valve and three coulometric array cell modules (each with four graphite working electrodes). The flow diagram for the analysis of monoamines, metabolites and drugs as well as derivatized amino acids has previously been described [16]. Briefly, analytes were separated on two stainless steel 150 x 4.6 mm NBS columns containing 5 µ M.S. Gel C18 (ODS particles having 120A pore size; distributed by ESA Inc.). Two detector arrays were used. Monoamines, metabolites and drugs were measured on eight electrodes (0 to 490 mV, 70 mV increments; vs. palladium reference); the OPA/ßME amino acid derivatives were measured using four electrodes set at 250, 450, 550 and 650 mV, respectively. The columns, pulse dampeners and electrode arrays were housed in a temperature regulated compartment maintained at 33±0.1 °C. System control, data acquisition and analysis were performed with the CEAS software on an Epson 386 computer. Two twenty microliter aliquots of diluted perfusate were analyzed for each sample [16].

Derivatization Protocol.

Samples were derivatized automatically on the autosampler. The method was based on that of Donzanti and Yamamoto [17], modified by the addition of 10 µM EDTA to the borate buffer diluent. Fresh OPA stock solutions were prepared weekly (27 mg OPA was dissolved in 1 ml methanol; after the

addition of 5 µl of ßME, this solution was diluted to 10 ml with a borate/EDTA solution (0.1 M sodium tetraborate, pH 9.3; 10 µM EDTA)). The stock solution was stored at 4 ^{o}C and was protected from light. The OPA working solution was prepared daily by dilution of the OPA stock solution with borate/EDTA 1:3 (v/v). The working solution was placed in amber vials in the refrigerated autosampler (4 ^{o}C).

Mobile Phases

Monoamines, metabolites and drugs were eluted using mobile phase A, consisting of: 0.054 M monobasic sodium phosphate containing 1.24 mM heptane sulfonic acid (sodium salt)/methanol 92:8 (v/v), adjusted to pH 3.0 with HPLC grade phosphoric acid. Amino acid derivatives were eluted using mobile phase B, consisting of: 0.139 M dibasic sodium phosphate/ acetronitrile/ methanol - 71.9:3.1:25 (v/v/v), adjusted to pH 6.8 with HPLC grade phosphoric acid. A flow rate of 1.2 ml/min was used for both mobile phases.

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Standards

Individual monoamine and metabolite standard stock solutions (0.1 to 1.0 mg/ml) were prepared in 0.1 M PCA containing 10 µg/ml ascorbic acid and 10% methanol (v/v). Each solution was purged with nitrogen and stored at -80 ^{o}C (stable for several months). Amino acid stock solutions (1 mg/ml) were prepared in 50% methanol (v/v) and stored at -4 ^{o}C. An external standard mixture was used for the perfusate analysis containing the monoamine and metabolites at 5 pg/µl and the amino acids at 500 pg/µl prepared in acidified (0.1 M PCA) aCSF.

Individual drug standards (1 mg/ml) were prepared in water and stored at 4^{o}C with the exception of apomorphine and hydralazine which were dissolved in 50% methanol v/v containing 10 µg/ml ascorbic acid and were prepared on the day of analysis (also stored at 4 ^{o}C). Drug mixtures for analysis were prepared at final concentrations ranging from 10-100 pg/µl in 10 µg/ml ascorbic acid.

Data Analysis

Data were acquired and analyzed using the CEAS software version 4.0 (ESA, Inc.). Data manipulation was performed using LOTUS 1-2-3 (Lotus Corp., Cambridge, MA, USA) and GBSTAT (Dynamic Microsystems, Inc., Silver Spring, MD, USA) software.

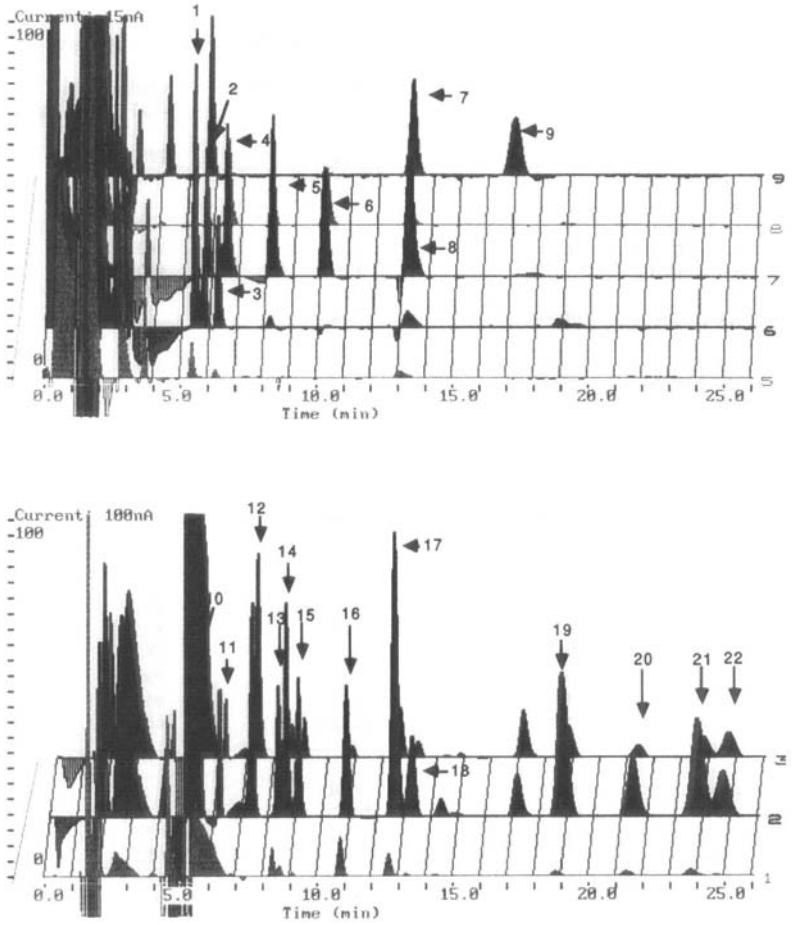

FIGURE I

Three dimensional chromatogram of the external standard mixture illustrating chromatographic and voltammetric resolution of each compound. The upper figure illustrates resolution of the monoamines and metabolites (100 pg on column) on electrodes 5 to 9 (0 to 280 mV; 70 mV increments) at a sensitivity of 15 nA full scale. The lower figure illustrates the resolution of derivatized amino acids (10 ng on column) on electrodes 1 to 3 (250, 450 and 550 mV respectively) at a sensitivity of 100 nA full scale. 1 - DA; 2 - 3-hydroxykynurenine; 3 - DOPAC; 4 - 5-hydroxytryptophan; 5 - 5HTOL; 6 - 5HIAA; 7 - 3MT; 8 - 5HT; 9 - HVA; 10 - ASP; 11 - GLU; 12 - ASN; 13 - HIS; 14 - SER; 15 - GLN; 16 - ARG; 17 - GLY; 18 - THR; 19 - TAU; 20 - ALA; 21 - GABA; 22 - TYR.

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TABLE I
Abbreviations, Approximate Retention Times And Oxidation Potenials For Analytes Discussed In Text

MONOAMINES AND METABOLITES	ABBREVIATIONS	APPROXIMATE R.T. (min)	OXIDATION POTENTIAL (mV)
3-Methoxytyramine	3MT	14.12	280
5-Hydroxyindole-acetic acid	5HIAA	10.78	140
5-Hydroxytryptamine	5HT	14.13	140
5-Hydroxytryptophan	5HTP	6.60	140
5-Hydroxytryptophol	5HTOL	8.75	140
Dopamine	DA	5.57	70
3,4-Dihydroxyphenyl-acetic acid	DOPAC	6.34	70
Homovanillic Acid	HVA	17.58	280
Norepinephrine	NE	2.51	70
AMINO ACIDS			
Alanine	ALA	21.69	450
Arginine	ARG	10.70	450
Asparagine	ASN	7.50	450
Aspartic acid	ASP	5.56	450
GABA	GABA	24.12	450
Glutamine	GLN	9.22	450
Glutamic acid	GLU	6.28	450
Glycine	GLY	12.75	450
Histidine	HIS	8.48	450
Serine	SER	8.76	450
Taurine	TAU	18.99	450
Threonine	THR	13.40	450
Tyrosine	TYR	25.02	450
DRUGS			
Apomorphine	APO	4.50	0
Hydralazine	H	12.50	0
Isoproterenol	ISO	8.90	70
Methoxamine	Mx	5.00	900
Morphine	M	11.10	280
Morphine-3-glucuronide	M3G	4.00	900
Phenylephrine	Phe	7.60	700

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RESULTS

External Standard Analysis

Two, three-dimensional chromatograms obtained from the analysis of the 18 component external standard are shown in Figure I. The abbreviation, retention time and oxidation potential for each compound are summarized in Table I. A representative chromatogram showing the resolution of the drug

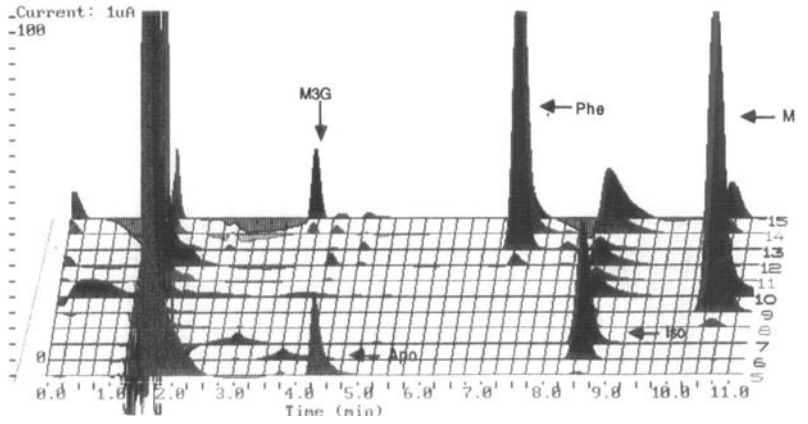

FIGURE II

Three dimensional chromatogram showing resolution of external standard drug mixture (1 ng/ul). An extended monoamine electrode array (twelve electrodes spanning 0 to 950 mV) was initially used to better examine each drug's electrochemical behavior. Output from electrodes 5 (0 mV) through 15 (800 mV) are shown at a gain of 1 μA. Analytical conditions are as described above.

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standard mixture is shown in Figure II. Analysis of aCSF did not show any contaminant peaks capable of interfering with measured analytes (data not shown). The assay was completed within 25 min.

Linearity And Limit Of Detection

Regression analysis of the peak height versus concentration of the monoamines, metabolites and amino acids demonstrated linear response over the range 0.25-20 pg/μl (correlation coefficient values (r) for 5HT, 5HIAA, DA and GABA were 0.9989, 0.9969, 0.9999 and 0.9975 respectively). Similarly, the drugs analyzed showed linear response over a concentration range of 0.5-500 pg/μl with r values for H, Iso, M, M3G and Phe of 0.9969, 0.9998, 0.9998, 0.9998 and 0.9998 respectively. Drug linearity (each point represents an n of 3) is presented in Figure III.

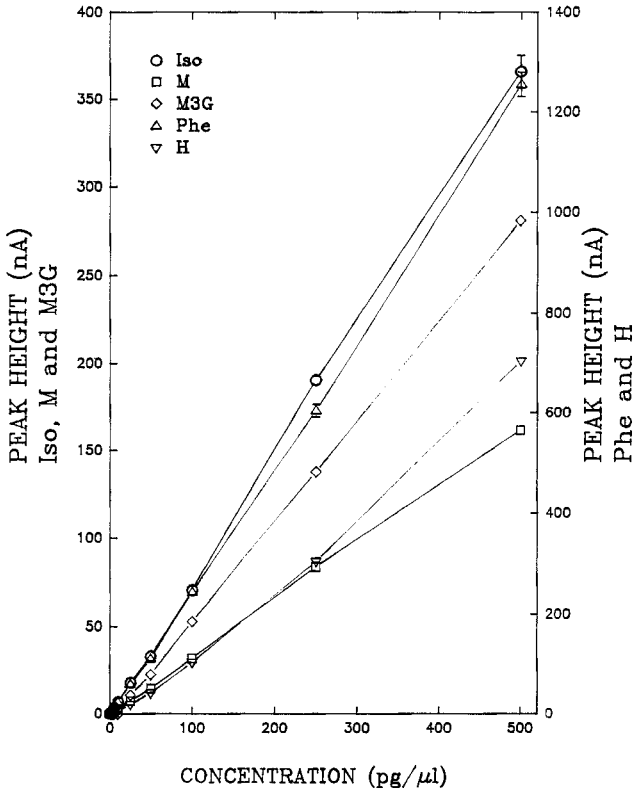

FIGURE III

Linearity of drug standards shown as peak height response (nA) over a concentration range of 0-500 pg/μl. Data presented as mean±SEM, n=3 for each data point.

The limit of detection (S/N ratio of 3:1) estimated from external standards for microdialysis perfusates was approximately 0.125 pg/μl for the monoamines and 0.75 pg/μl for the amino acids. The limit of detection (pg/μl) for H, Iso, M, M3G and Phe were: 0.65, 0.28, 1.25, 10.65 and 0.215 respectively.

Endogenous Metabolite Levels

The basal levels (in pg/collection, presented as mean±SEM) and ratio accuracies of monoamines, metabolites and amino acids in both striatal (n=3-6) and hippocampal (n=3) microdialysate samples are

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TABLE II
Basal Striatal and Hippocampal ECF Analyte Levels

	Basal Striatal Levels		Basal Hippocampal Levels	
Monoamines and Metabolites	pg/Collection Mean ± SEM (n = 3-6)	Typical "Ratio Accuracy"	pg/Collection Mean ± SEM (n = 3)	Typical "Ratio Accuracy"
3MT	54 ± 8.6	0.98	ND	--
5HIAA	2880 ± 585	0.99	1530 ± 63	0.99
5HT	ND	--	19.4 ± 11	0
5HTOL	ND	--	ND	--
DA	51.8 ± 6.8	0.99	63. ± 0.5	0.96
DOPAC	12645 ± 1485	0.70	259 ± 47	0.95
HVA	13905 ± 225	0.79	117 ± 14	0.95
NE	ND	--	ND	--
Amino Acids				
ALA	13095 ± 450	0.95	7695 ± 1215	0.98
ARG	14355 ± 1305	0.94	10170 ± 675	0.93
ASN	3420 ± 225	0	3510 ± 90	0
ASP	2835 ± 585	0	8730 ± 90	0.23
GABA	383 ± 32	0	293 ± 45	0
GLN	318195 ± 22860	0.91	21105 ± 4680	0.62
GLU	12870 ± 540	0.93	4860 ± 270	0.94
GLY	16425 ± 4590	0.93	5850 ± 765	0.75
HIS	11115 ± 495	0.91	ND	0.60
SER	21105 ± 1980	0.99	10395 ± 225	0.96
TAU	12501 ± 945	0.94	8973 ± 270	0.77
THR	21780 ± 3555	0.96	16470 ± 405	0.99
TYR	1800 ± 45	0.98	5625 ± 270	0.98

ND = Not detectable

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presented in Table II. More than 50-70 additional peaks were found in the microdialysis samples but could not be identified or quantitated due to the lack of appropriate external standards.

The effects of infusion with either H or NPr on striatal analyte levels calculated as percent of baseline (mean of three consecutive basal samples) at 20 and at 180 min are summarized in Table III. As shown in Figure IV, H caused an almost immediate 40% decrease in BP while increasing ECF DA levels 220 fold and decreasing those of DOPAC and HVA 80-90% (upper graph); there was also a rapid 30-120 fold increase in amino acid levels (lower graph). Although NPr also stimulated DA release by approximately 100 fold, decreased DOPAC and HVA levels 40-50% and decreased BP 30% (Figure V, upper graph), the effect of NPr on amino acid levels (Figure V, lower graph) was markedly different to that of H. The passage of H through the BBB reached a maximum of 9 pg/µl (405 pg/collection; uncorrected for in vitro

TABLE III
Effects of Hydralazine Or Nitroprusside On Striatal Analyte Levels

Monoamines and Metabolites	Hydralazine % Of Baseline At 20 min	Hydralazine % Of Baseline At 180 min	Nitroprusside % Of Baseline At 20 min	Nitroprusside % Of Baseline At 180 min
3MT	(8.2)	(15.62)	203	262
5HIAA	93	17	99	41
5HT	ND	ND	ND	(1.11)
5HTOL	ND	(1.2)	ND	ND
DA	22374	3427	1394	10350
DOPAC	101	20	108	51
HVA	77	12	95	31
NE	ND	ND	ND	ND
Amino Acids				
ALA	257	647	105	509
ARG	89	100	84	75
ASN	130	241	91	163
ASP	651	1929	95	1029
GABA	3468	25796	133	6044
GLN	83	71	78	50
GLU	472	3466	102	2030
GLY	217	915	78	249
HIS	91	131	84	104
SER	200	255	80	102
TAU	525	3398	77	1432
THR	139	172	87	139
TYR	114	248	111	260

ND = Not detectable

The numbers in paranthesis are given in pg/µl (not as a percentage change of baseline) as basal striatal levels of 3MT and 5HTOL, and basal hippocampal levels of 5HT were below the detection limit.

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recovery) 40 min after its peripheral administration and is presented in Figure VI. NPr could not be detected using this method. Infusion of saline (i.v.) had no effect on any of the measured analytes (data not shown).

The peripheral administration of M had little effect on hippocampal metabolite levels with the exception of GABA and GLY which showed marked decreases (presented as % change of baseline at 20min and 80 min. See Table IV). The passage of M through the BBB reached a maximum of 12.6 pg/µl (567 pg/collection; uncorrected for in vitro recovery) 40 min after its i.p. administration and is shown in Figure VII. M3G could not be measured centrally after peripheral administration of M. Administration of saline (i.p.) had no effect on any of the measured analytes (data not shown).

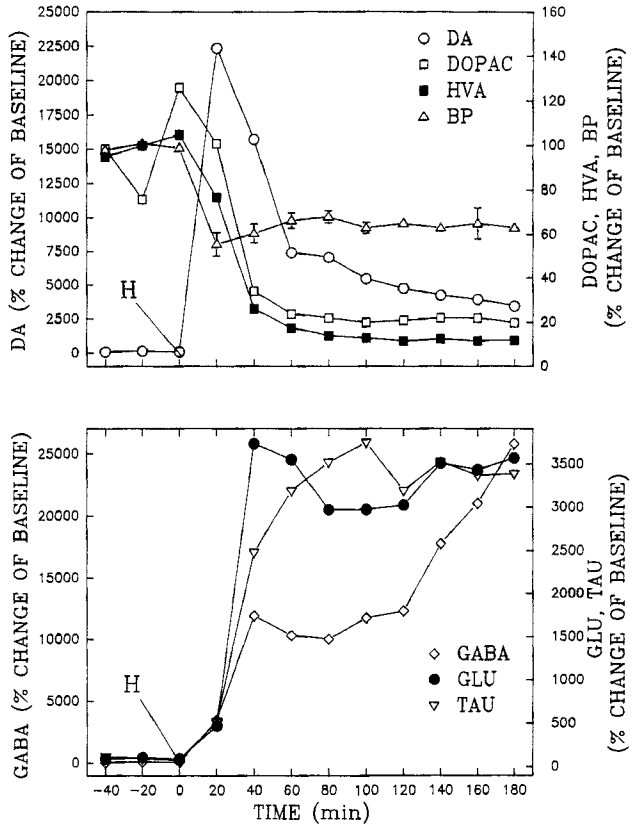

FIGURE IV

The effects of H (10 mg/kg i.v. given as bolus at t=0 min) on striatal ECF levels of analytes and blood pressure in the urethane anesthetized rat. Data are presented as % change of basal levels (100%) vs. time (min). The upper figure shows the response of DA (left vertical axis) and of DOPAC, HVA and BP (right vertical axis) to H infusion. The lower figure shows the response of GABA (left vertical axis) and of GLU and TAU (right vertical axis) to H infusion.

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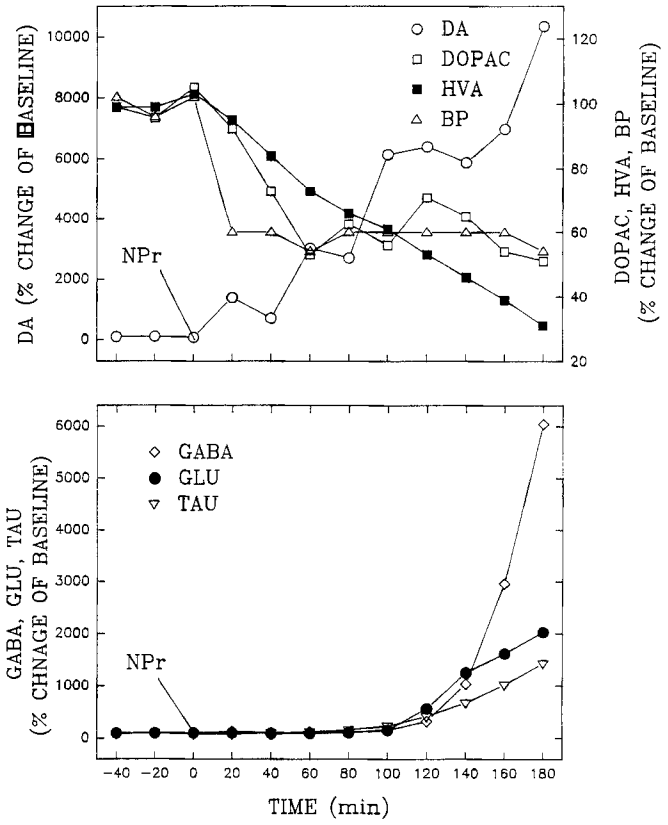

FIGURE V

The effects of NPr (0.06-0.3 mg/min/kg i.v.; infusion started at t=0 min) on striatal ECF levels of analytes and blood pressure in the urethane anesthetized rat. Data are presented as % change of basal levels (100%) vs. time (min). The upper figure shows the response of DA (left vertical axis) and of DOPAC, HVA and BP (right vertical axis) to NPr infusion. The lower figure shows the response of GABA, GLU and TAU to NPr infusion.

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TABLE IV
Effects Of Morphine On Hippocampal ECF Analyte Levels

Monoamines and Metabolites	% Of Baseline 20 min	80 min	Amino Acids	% Of Baseline 20 min	80 min
3MT	ND	ND	ALA	67	63
5HIAA	85	97	ARG	81	81
5HT	98	30	ASN	94	105
5HTOL	ND	ND	ASP	ND	ND
DA	150	121	GABA	78	52
DOPAC	103	133	GLN	57	56
HVA	97	41	GLU	110	91
NE	ND	ND	GLY	66	16
			HIS	85	87
			SER	80	78
			TAU	86	96
			THR	80	75
			TYR	88	73

ND = Not detectable

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DISCUSSION

The results presented here indicate that this method may be used for the routine measurement of a variety of analytes in microdialysis samples. Multiple analyte measurement in a single analytical run should minimize experimental error induced by excessive sample handling, sample instability, as well as differences in extraction efficiency and instrument calibration. Additionally, data is obtained from a single animal overcoming potential errors caused by inter-animal variability. The coulometric array also allows for on-line resolution of co-eluting peaks (e.g. 5HT and 3MT). Since resolution is obtained in two dimensions (chromatographic and voltammetric), many components may be selectively determined within a single run. Response "ratio accuracies" (a simple mathematical comparison of the respective hydrodynamic voltammograms obtained from the external standard and the sample unknown [18,19]) provide an objective index of peak purity and confirmation of peak identity. The closer this number is to unity the greater the certainty of peak purity. A response "ratio accuracy" of zero represents a situation where there is only sufficient signal to quantitate on the highest responding electrode.

Our method is also capable of measuring a variety of drugs and can be used to examine their passage through the BBB. This is the first report of the direct measurement of the passage of H into the brain. Penetration is unlikely the result of H's unrestricted access through damaged areas of the BBB caused by probe insertion as the BBB is known to rapidly reseal after the initial trauma [14,20].

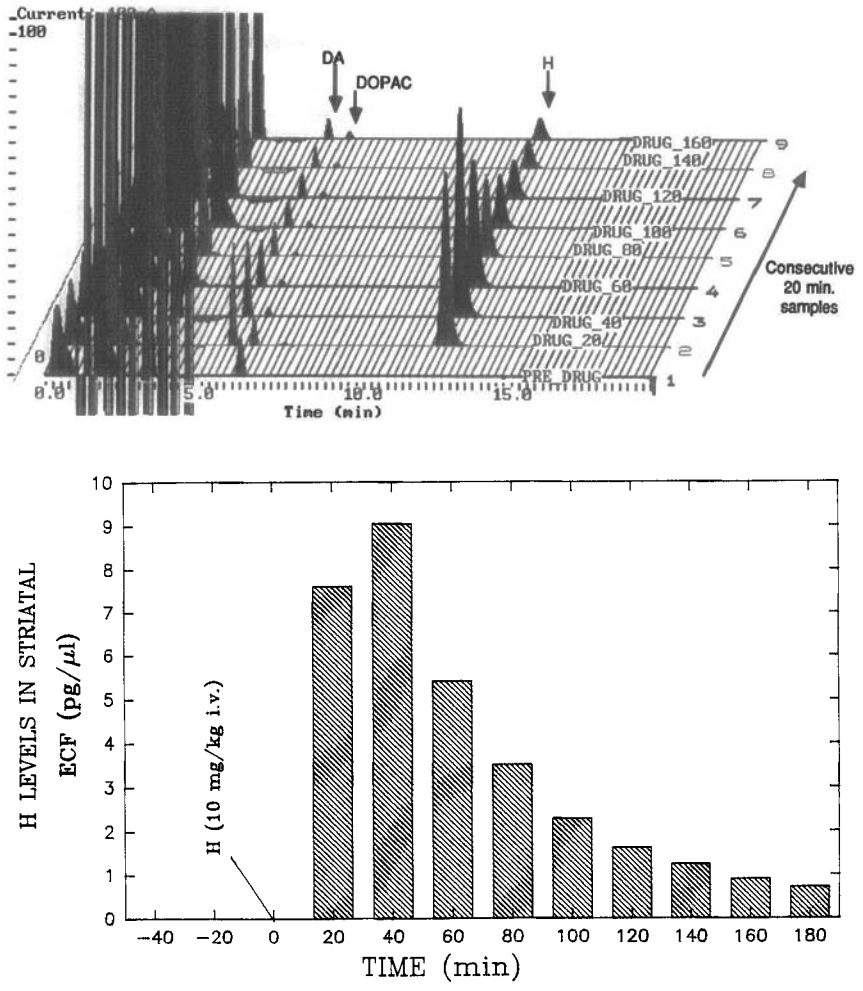

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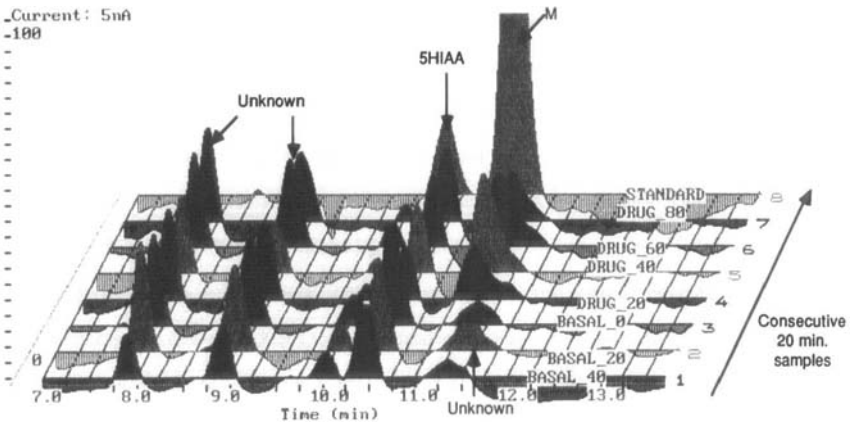
FIGURE VI

The simultaneous measurement of the passage of H through the BBB and its effect on striatal DA and DOPAC levels is illustrated in the upper figure. The response of electrode 5 (0 mV; 100 nA sensitivity full scale) is shown for consecutive 20 min samples, before and after H infusion (10 mg/kg i.v. given as a bolus at "Pre Drug"; #1). Striatal levels of H reached a maximum of 9 pg/μl 40 min after its administration - lower figure.



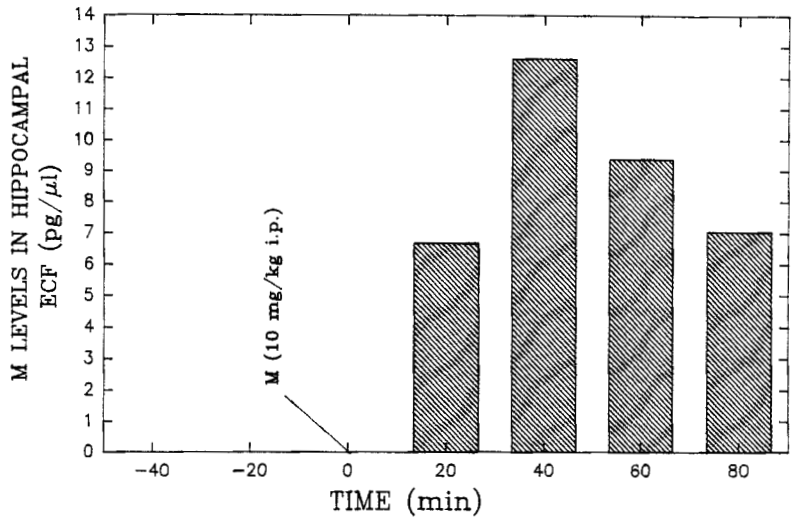


FIGURE VII

The simultaneous measurement of the passage of M through the BBB and its effect on hippocampal levels of 5HIAA and unknowns is illustrated in the upper figure. The response of electrode 9 (280 mV; 5 nA sensitivity full scale) is shown for consecutive 20 min samples, before and after M infusion (10 mg/kg i.p. given as a bolus at "Basal - 0") as well as for M external standard (100 ng/ml; #8). An unknown endogenous analyte eluted just after the M peak, but did not interfere with M quantification. Hippocampal levels of M reached a maximum of 12.6 pg/μl 40 min after its administration - *lower figure.*

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Drugs used in this study were chosen due to their use in ongoing research. Of all the drugs measured the assay was least sensitive for measurement of M3G. This low M3G sensitivity may be due to the fact that its oxidation occurred at +900 mV, a potential with more noise and at which potential many endogenous compounds also oxidize. Apo was the most difficult compound to measure reliably due to its inherent instability and tendency to auto-oxidize. The inclusion of ascorbic acid in the diluent solution and EDTA (1 μM) in the mobile phase improved Apo's stability to some degree. Problems caused by system heavy metal catalyzed oxidation [21] were diminished by replacing the majority of stainless steel in contact with the fluid path with inert PEEK components wherever possible. Several other drugs were also examined including clonidine, MPP^+, quinpirole and SKF-38393. However, these could not be measured using the current system and their detection awaits further method development.

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Basal analyte levels were generally in good agreement with values previously published for both the striatum [13, 22-24] and the hippocampus [22,25-27] when differences in probe membrane surface area, perfusion flow-rate, anesthetic and animal model are taken into consideration. Although tissue levels of NE can be easily quantitated using this method, due to the proximity of the NE peak to the void disturbance and low NE levels typically found in hippocampal microdialysis samples (ca. 1-10 pg/collection [26,28] accurate measurement of NE levels in these samples is difficult. The elution of NE can be delayed by reducing the methanol concentration of mobile phase A to 4% which then enables NE to be quantitated without void interference. Although 5HT is thought to act as a neurotransmitter in both the striatum [27] and hippocampus [29], over-estimation of its ECF level can easily occur as a result of simultaneous release from lysed platelets when surrounding tissue is damaged [30]. A more appropriate experiment would use the awake-animal model. This would permit at least 12 hrs between the time of probe insertion and the start of ECF analysis by which time neurotransmitter levels should more closely reflect basal conditions [31-33]. DA has previously been reported to be released from hippocampal synaptosomes [34] and has been recently measured in hippocampal ECF using microdialysis [35]. However, whether it acts as a neurotransmitter per se, or represents a precursor pool which is co-released with NE is presently being addressed [36].

Our data supports previous reports that drug induced hypotension can markedly increase striatal DA release while decreasing its catabolism [37]. Although both the antihypertensive H and NPr promote DA release to the same extent, the differences in the time course of DA release and the patterns of amino acids detected argues against a common mechanism of action. It is unlikely that H's effect can be

entirely explained through its weak monoamine oxidase inhibitor ability (administration of pargyline elevated ECF DA levels to a much lesser extent) or to ischemia. Ischemia would be expected to elevate 5HT to detectable levels [38,39] and to promote monoamine release in other brain regions. H has previously been reported to elevate DA and NE by only 2-3 fold in the hippocampus and has been shown to have no significant effect on their ECF levels in the pre-frontal cortex [40]. It is also doubtful that H is acting through a non-specific toxic mechanism as its effects on both DA and BP levels can be blocked by the co-administration of the $alpha_1$ adrenergic agonist Mx (a situation which would not be expected to interfere with the passage of H through the BBB) [37].

In a previous report F. Matos and colleagues [12] examined levels of M and its effect on 5HT, 5HIAA and HVA in the ECF from several brain regions as well as in CSF. Our data complement their findings and our method permits a more detailed examination of the effects of M on a variety of other compounds including several amino acids. Although M had some effect on most of the amino acids it markedly decreased the levels of the inhibitory amino acids, GABA and GLY, by 48 and 84% respectively. Interestingly, although M has previously been reported to act at opioidergic receptors in the substantia nigra and striatum and to involve interactions with both dopaminergic and GABA-ergic neurons [41], no reports of its action on hippocampal ECF levels of GABA could be found. Similarly, the peripheral administration of M has also been shown to decrease GLY levels in mouse brain homogenates [42] but its effect on ECF levels of GLY has not been previously demonstrated. In humans, M3G is the principal catabolite and potent morphine antagonist (M6G is only a minor metabolite) [43]. Hippocampal ECF levels of M3G could not be measured after peripheral M administration, suggesting either that formation of this conjugate is not the major route of catabolism in rat brain or, that if formed in the periphery, does not pass through the BBB.

In conclusion, our results demonstrate the potential of coupling microdialysis to HPLC array-ECD analysis for measuring drug levels in the ECF obtained from discrete brain areas with simultaneous determination of monoamines, metabolites and amino acids. The use of the awake, freely-moving animal will allow for the study of drug-induced changes in behavior concomitantly with the continuous monitoring of drug levels in discrete brain regions. It is hoped that different methods can be similarly linked using the switching valve so that different analytes may be measured simultaneously in the same sample (e.g. amino acids and derivatized drugs).

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ACKNOWLEDGMENTS

The authors would like to thank Sheri Cottreau for help with preparation of the figures.

REFERENCES

1. F. Moroni, G. Pepeu, (1984) "The Cortical Cup Technique," in Measurement of Neurotransmitter Release In Vivo, C.A. Marsden, ed., John Wiley, Chicester, 1984, pp. 63-79.

2. A. Philippu, (1984) "Use of Push-pull Cannulae to Determine Release of Endogenous Neurotransmitters in Distinct Brain Areas of Anesthetized and Freely Moving Animals," in Measurement of Neurotransmitter Release In Vivo, C.A. Marsden ed., John Wiley, Chicester, 1984, pp. 3-37.

3. T.L. Yaksh, "Spinal Superfusion in Rat and Cat," in Measurement of Neurotransmitter Release In Vivo, C.A. Marsden ed., John Wiley, Chicester, 1984, pp. 107-124.

4. Y.L. Hurd, J. Kehr, U. Ungerstedt U. J. Neurochem., 51: 1314-1316 (1988)

5. K. Sabol, C. Freed, J. Neurosci. Methods, 24: 163-168 (1988)

6. L. Stahle, S. Segersvard, U. Ungerstedt, Eur. J. Pharmacol., 185: 187-193 (1990)

7. E.P. Wala, W.R. ., Martin, J.W. Sloan, Psychopharmacol. Berl., 105: 535-540 (1991).

8. W. Maruyama, D. Nakahara, M. Ota, T. Takahashi, A. Takahashi, T. Nagatsu, T. and M. Naoi, J. Neurochem., 59: 395-400 (1992)

9. U. Ungerstedt, "Measurement of Neurotransmitter Release by Intracranial Dialysis," in Measurement of Neurotransmitter Release In Vivo, C.A. Marsden ed., John Wiley, Chicester, 1984, pp. 81-105.

10. U. Ungerstedt, "Introduction to Microdialysis," in Microdialysis in The Neurosciences. Techniques in The Behavioral and Neural Sciences, Vol. 7. T.E Robinson and J.B. Justice Jr. eds., Elsevier, London, 1991, pp. 3-22.

11. H. Rollema, B.H.C. Westerink, W.J. Drijfhout, Monitoring Molecules in Neuroscience, Krips Repro, Meppel, The Netherlands, 1991.

12. F.F. Matos, H. Rollema, A.I. Basbaum, J. Neurochem., 58, 1773-1781 (1992)

13. B. Moghaddam, B. S. Bunney, J. Neurochem., 53, 652-654 (1989)

14. H. Benveniste, A.J. Hansen, "Practical Aspects of Using Microdialysis for Determination of Brain Interstitial Concentrations" in Microdialysis in The Neurosciences. Techniques in The Behavioral and Neural Sciences, T.E Robinson and J.B. Justice Jr., eds., Vol. 7. Elsevier, London, 1991, pp. 81-100

15. P.F. Morrison, P.M. Bungay, J.K. Hsiao, I.N. Mefford, K.H. Dykstra, R.L. Dedrick, "Quantitative Microdialysis," in Microdialysis in The Neurosciences. Techniques in The Behavioral and Neural Sciences, T.E Robinson, J.B. Justice Jr. eds., Vol. 7. Elsevier, London, 1991, pp. 47-80.

16. P. Gamache, E. Ryan, C. Svendsen, K. Murayama, I.N. Acworth, J. Chromatog., 614, 213-220 (1993)

17. B.A. Donzanti, B.K. Yamamoto, Life Sci., 43: 913-922 (1988)

18. W.R. Matson, P. Langlais, L. Volicer, P.G. Gamache, E. Bird, K.A. Mark, Clin. Chem., 30: 1477-1488 (1984)

19. J.A. Wolff, L.J. Fisher, L. Xu, H.A. Jinnah, P.J. Langlais, P.M. Luvone, K.L. O'Malley, M.B Rosenberg, S. Shimohama, T. Friedmann, F.H. Gage, Proc. Natl. Acad. Sci., 86: 9011-9014 (1989)

Downloaded At: 08:08 25 January 2011

20. U. Tossman, U. Ungerstedt, Acta Physiol. Scand., 128: 9-14 (1986)

21. J. X-. Huang, J.D. Stuart, W.R. Melander, C. Horvath, J. Chromatog., 316: 151-161 (1984)

22. U. Tossman, G. Jonsson, U. Ungerstedt, Acta Physiol. Scand., 127: 533-545 (1986)

23. I.N. Acworth, M.J. During, R.J. Wurtman, Brain Res. Bull., 21: 473-477 (1988)

24. J. Kehr, U. Ungerstedt, J. Neurochem., 51: 1308-1310 (1988)

25. A. Hamberger, B. Nystrom, Neurochem. Res., 9: 1181-1191 (1984)

26. E.D. Abercrombie, J.M. Finlay, "Monitoring Extracellular Norepinephrine in Brain Using In Vivo Microdialysis and HPLC-EC," in Microdialysis in The Neurosciences. Techniques in The Behavioral and Neural Sciences, T.E Robinson and J.B. Justice Jr., eds. ,Vol. 7. Elsevier, London, 1991, pp. 253-274.

27. A. Adell, A. Carceller, F. Artigas, J. Neurochem., 56: 709-712 (1991)

28. E.D. Abercrombie, M.J. Zigmond, J. Neurosci., 9: 4062-4067 (1989)

29. P. Kalen, R.E. Strecker, E. Rosengren, A. Bjorklund, J. Neurochem., 51, 1422-1435 (1988)

30. J.R. Fozard, The Peripheral Actions of 5-Hydroxytryptamine, Oxford University Press, New York 1989.

31. B.H.C. Westerink, M.H.J. Tuinte, J. Neurochem., 46: 181-185 (1986)

32. B.H.C. Westerink, J.B. De Vries, J. Neurochem., 51: 683-687 (1988)

33. D.M. Camp, T.E. Robinson, J. Neurochem., 58: 1706-1715 (1992)

34. M. Verhage, W.E.J.M. Ghijsen, F. Boomsma, F.H Lopes da Silva, J. Neurochem., 59: 881-887 (1992)

35. C.S. Biggs, B.R. Pearce, L.J. Fowler, P.S. Whitton, J. Neurochem., 59: 1702-1708 (1992)

36. K.C. Gariepy, B.A. Bailey, J. Yu, T.J. Maher, I.N. Acworth, J. Chromatog., (submitted).

37. J. Yu, K. Gariepy, I.N. Acworth, T.J. Maher, Poster #197.10, pp. 460. Society for Neuroscience, Anaheim, California, 1992.

38. G. Damsma, D.P. Boisvert, L.A. Mudrick, D. Wenkstern, H.C. Fibiger, J. Neurochem., 54, 801-808 (1990)

39. G.S. Sarna, T.P. Obrenovitch, T. Matsumoto, L. Symon, G. Curzon, J. Neurochem., 55: 937-940 (1990)

40. J. Yu, I.N. Acworth, K. Gariepy, T.J. Maher, T. The Federation of American Societies for Experimental Biology, New Orleans, 1993.

41. K. Kamato, Japan J. Pharmacol., 45, 439-447 (1987)

42. P. Stern, S. Catovic, N. Filipovic, Pharmacol., 10: 97-108 (1973)

43. G.J. Mulder, Annu. Rev. Pharmacol. Toxicol., 32: 25-49 (1992)

Received: June 6, 1993
Accepted: August 2, 1993

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