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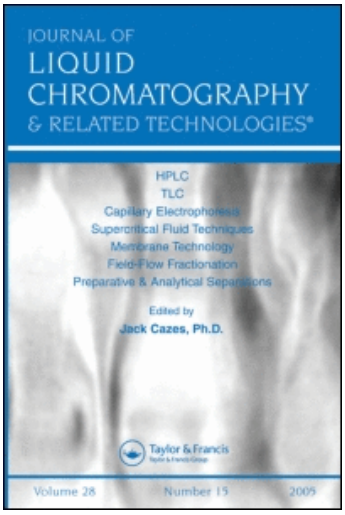

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Determination of Spinal Cord Monamines and Metabolites Using Three Micron Columns and Dual Electrochemical Detection

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DETERMINATION OF SPINAL CORD MONAMINES AND METABOLITES USING THREE MICRON COLUMNS AND DUAL ELECTROCHEMICAL DETECTION

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ABSTRACT

A method, based on liquid chromatography with electrochemical detection, for the simultaneous determination of monoamines (norepinephrine, dopamine, serotonin) and their major metabolites (4-hydroxy-3-methoxyphenethylene glycol, homovanillic acid, 5-hydroxy-indole-3-acetic acid) in small segments of spinal cord (ca. 30-70 mg tissue wet weight) is presented. The combination of two recent technological advances; short reversed-phase columns packed with 3 micron diameter particles and parallel dual-electrode amperometric detectors, has allowed the resolution and quantitation of a large number of electroactive species in a relatively short time and with a high degree of confidence regarding peak purity. Aspects of the performance of the method are discussed. The calculated concentrations of the analytes of interest are presented, and compared to previously reported values obtained using other analytical techniques.

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INTRODUCTION

Liquid chromatography with electrochemical detection has become the method of choice for the routine determination of catecholamines, indoleamines and their precursors and metabolites in biological tissues and fluids (1). Recent advances in column technology, i.e. the development of three micron particle packings, has allowed the separation of nearly the complete range of central nervous system (CNS) monoamines and related compounds in one short chromatographic step (2). The development of dual-electrodes for electrochemical detection has allowed a more selective determination of CNS monoamines in filtered supernatants of brain homogenates and sonicates by virtue of their ability to detect these compounds at two separate applied potentials (3).

Here, we report the combination of these two technological advances for the determination of norepinephrine (NE), 4-hydroxy-3-methoxyphenylethylene glycol (MHPG), dopamine (DA), homovanillic acid (HVA), serotonin (5-HT) and 5-hydroxy-indole-3-acetic acid (5HIAA) in filtered supernatants of spinal cord sonicates. The concentration and estimated utilization (ratio of metabolite to transmitter) values in rabbits are reported as are some performance parameters of the method.

EXPERIMENTAL

Reagents

The following compounds were purchased from Sigma (St. Louis, MO, U.S.A.): NE, MHPG, DA, HVA, 5-HT, 5HIAA, dihydroxybenzylamine

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(DHBA), epinephrine (E), 3,4-dihydroxyphenylacetic acid (DOPAC), 3,4-dihydroxylphenylglycol (DHPG), 3,4-dihydroxymandelic acid (DHMA), normetanephrine (NM), metanephrine (MN), 3-methoxytyramine (3-MT), tyrosine (Tyr), tryptophan (Trp), 5-hydroxytryptophan (5-HTP), indole-3-acetic acid (IAA), melatonin (M), vanilmandelic acid (VMA), L, β, 3,4-dihydroxyphenylalamine (L-DOPA), L, α-methyl, β, 3,4-dihydroxyphenylalanine (αCH_3-DOPA), 6-hydroxydopamine (6-OH-DA), 4-methoxy-3-hydroxyphenethanol (MOPET), 4-hydroxy-3-methoxyphenethylamine (MTA), ascorbic acid (AA) and uric acid (UA). Stock solutions of these compounds were prepared in 1-5 mg/ml aliquots in 0.1 M $HClO_4$, 0.05 M $HClO_4$ (all indoleamines) water (neutral compounds) or pH 4.3 phosphate buffer (HVA), and stored at -90° C. Standard solutions of the analytes of interest (NE, MHPG, DA, HVA, DOPAC, E, 5-HT and 5HIAA) were prepared weekly in 0.1 M $HClO_4$ at a concentration of 100 ng/ml. Working solutions were diluted daily from these standard solutions. The working internal standard solution (DHBA) was prepared daily at a concentration of 50 ng/ml in 0.05 M $HClO_4$ from a 200 ng/ml 0.1 M $HClO_4$ standard solution. All dilutions and all mobile phases were prepared from HPLC grade water and methanol (Fisher Scientific, Fairlawn, NJ, U.S.A.). All other chemical were of the certified A.C.S. grade.

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Chromatographic System

An M-45 dual piston pump (Waters Assoc., Milford, MA, U.S.A.), Rheodyne 7125 injection valve fitted with a 20 μl loop (Rheodyne, Berkely, CA, U.S.A.), Supelcosil LC-18 reversed phase

column 7.5 cm x 4 mm i.d., 3 micron particles, a C_{18} Supelguard guard column, 2.0 cm x 4 mm i.d., 5 micron particles (Supelco Inc., Bellefonte, PA, U.S.A.), MF 1000 glassy carbon dual electrode bottom with a low dead volume stainless top controlled by two LC-4B amperometric detectors each equipped with a 3-pole Butterworth active filter circuit (Bioanalytical Systems, W. Lafayette, IN, U.S.A.) and dual-pen potentiometric recorder (Houston Inst., Austin, TX, U.S.A.) made up the chromatographic system. Besides the use of the stainless-steel detector top and active electronic filter, other modifications for high-speed analysis included the use of a low dead volume direct-connect coupler (Alltech Assoc., Waukegan, IL, U.S.A.) to join the guard and analytical columns; a 3 cm length of 0.005 in i.d. tubing equipped with plastic fingertight fittings (Upchurch Sci., Oak Harbor, WA, U.S.A.) connecting the analytical column to the detector, and a 3 cm length of 0.01 in tubing connecting the injection valve to the guard column. The total length from injector outlet to detector inlet was approximately 21 cm.

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Spinal Cord Samples

White New Zealand rabbits (2.0-2.5 kg) were sacrificed with an overdose of sodium pentobarbital (40 mg/kg). The animals were part of a larger study on the effects of impaction-injury on spinal cord monoamine content and utilization which will be reported elsewhere. Prior to sacrifice, a sterile laminectomy was performed over segments T_9-T_{11}. This report deals with data from laminectomized rabbits only (N=14). These animals were sacrificed

30 min, 1 hour, 4 hours, and 5 hours after laminectomy, at which times 6 x 5 mm samples of the spinal cord corresponding to segments T_9-T_{11} (two samples per segment) were removed, weighed and stored at -90°C for up to 2 weeks prior to analysis. All manipulations were performed at the same time of day. Furthermore, all animals were placed on identical light-dark cycles for two weeks prior to sampling. These procedures minimized the contribution of circadian variation in monoamine content to the results.

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Sample Preparation

Spinal cord segments (N=84, 31.40-71.90 mg wet weight, mean ± S.E.M. = 50.43 ± 1.12 mg) were thawed and 0.2 ml of the working internal standard solution (50 ng DHBA/ml 0.05 M $HClO_4$) was added to each. Samples were then sonicated on ice for 5 sec using a Heat Systems sonicator (Model W185, Heat Systems-Ultransonics, Inc., Plainview, NY, U.S.A.) at a setting of 4. The combination of freeze-thawing and sonication resulted in efficient rupturing of cell membranes and subsequent liberation of amines into the supernatant. The sonicated samples were then centrifuged at 10,000 x g for 10 min at 4°C. The resulting supernatants were filtered using centrifugal microfilters (Bioanalytical Systems) fitted with 0.20 micron nylon membranes. Twenty µl portions of these filtrates were injected in duplicate onto the chromatograph. Standards consisted of the compounds of interest in 0.05 M $HClO_4$ containing 50 ng/ml DHBA and at three concentrations covering the range of sample values.

Chromatographic Analysis

Initially, two separate mobile phase systems were employed in order to optimize method selectivity. These consisted of: anhydrous citric acid, A-7.0 mM, B-2.4 mM; dibasic sodium phosphate heptahydrate, A-11.5 mM, B-5.1 mM; sodium octylsulfate (ion-pairing reagent), A-0.43 mM; B-0.34 mM; disodium EDTA (metal chelator) A-1.3 mM, B-1.3 mM and diethylamine, (to mask unbonded silanol groups and reduce peak tailing) A-0.12%, B-0.26%, used as supplied from Sigma. The pH of each mobile phase was adjusted with concentrated phosphoric acid (HPLC grade, Fisher Sci.) to: A-3.5, B-5.0. Mobile phases were filtereed through 0.22 micron membranes (Millipore, Bedford, MA, U.S.A.), degassed in vacuo, maintained at 40-50°C and pumped at: A-2.2 ml/min, B-1.5 ml/min. Both systems were recycled for one week periods after which the entire chromatograph was flushed with water flowed by a 1:1 mixture of water:methanol. In addition, the pump was passivated monthly with 10% nitric acid. The settings used for the amperometric detectors were +0.65 V (or +0.70 V) vs Ag/AgCl at 0.1 Hz and +0.80 V vs Ag/AgCl at 0.5 Hz. The transducer was placed in the dual-parallel mode.

Twenty samples were assayed in duplicate using the two separate mobile phase systems. Since the values obtained were identical, mobile phase A only was used for subsequent determinations since the compounds of interest could be determined in a much shorter time (17 min vs 26 min). In all, four separate procedures were used to confirm peak identity: co-elution with

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authentic standards, peak height ratios at two applied potentials, duplicate determinations in two separate mobile phase systems, and exclusion of the twenty-one possible electroactive interferences listed under reagents.

RESULTS AND DISCUSSION

The retention times for the twenty-seven compounds tested in each mobile phase are listed in Table 1. Note that the six analytes of interest are resolved from the twenty-one possible interferences tested in each system. Sample chromatograms of a working external standard solution and spinal cord sample are illustrated in Figure 1. Mobile phase A, the system of choice, was employed. Note the excellent peak symmetry and low peak volume in spite of the use of 20 µl injections. We have found negligible to no band broadening with 20 µl injections as opposed to 5 µl injections. The use of 20 µl injections allowed us to quantitate the very low concentrations of DA and HVA in spinal tissue. Reinhard and Perry have employed injection volumes up to 70 µl on three micron columns with no loss of resolution or theoretical plate height reported (4). It is likely, however, that smaller injection volumes (ca. 5 µl or less) would be more advantageous for early eluting peaks, such as NE and MHPG, which occupy as little as 300 µl total volume at a flow rate of 2.2 ml/min. This factor, along with the electronic filter setting employed, is particularly crucial for fast-eluting peaks. For instance, note that in the chromatogram of spinal tissue in

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TABLE 1

Compound	Retention Time (min) Mobile Phase A	Mobile Phase B
AA	0.46	0.30
UA	0.50	0.30
DHMA	0.67	0.50
DHPG	0.75	1.25
*Tyr	0.75	0.50
VMA	1.00	0.80
NE	1.16	1.78
MHPG	1.33	2.00
E	1.92	2.33
L-DOPA	2.15	1.50
6-OH-DA	2.45	4.00
DHBA	2.67	4.35
DOPAC	3.00	1.47
NM	3.50	6.83
DA	4.67	7.67
MN	5.75	9.00
$^{\alpha}CH_3$DOPA	5.92	7.15
5HTP	6.13	4.00
5HIAA	6.58	3.60
MOPET	7.67	8.43
HVA	9.50	3.90
3-MT	15.45	>30
5-HT	16.00	25.83
MTA	23.67	>30
*Trp	>30	>30
*M	>30	>30
*IAA	>30	>30

*Not Detected at applied potentials employed.

Figure 1B, the peak corresponding to MHPG in the lower trace (+0.80 V, 0.5 Hz) is barely resolved from a larger peak eluting about 8 sec. later. The upper trace, which is more highly filtered (+0.65 V, 0.1 Hz) does not resolve a very small peak for MHPG from this interference at all. Note that in the standard chromatogram in Figure 1, the peak height of MHPG at +0.65 V is

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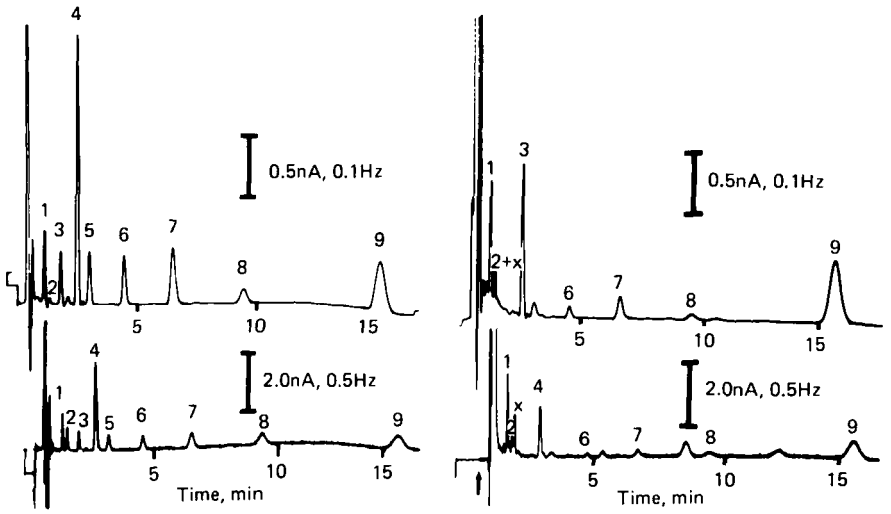

FIGURE 1. Sample dual-parallel chromatograms using the present method and mobile phase A (see text) of, A: working external standard solution (injected amounts); 1-NE (250 pg), 2-MHPG (250 pg), 3-E (250 pg) 4-DHBA (1 ng), 5-DOPAC (250 pg), 6-DA (250 pg), 7-5-HIAA (375 pg), 8-HVA (250 pg), 9-5-HT (750pg). B: spinal cord tissue sonicate. Peak identification as in A. X-unknown peak. In each dual chromatogram, the upper trace was recorded at an applied detector potential of +0.65 V versus Ag/AgCl, the bottom trace at +0.80 V versus Ag/AgCl. All other calibrations as noted. Sample injected at arrow in lower trace which is displaced approximately 1 min ahead of upper trace. Elution front from lower trace masked for clarity.

very low. Thus, in order to obtain a peak height ratio for MHPG at two potentials, it would be necessary either to lower the injection volume or lessen the degree of electronic filtering in the upper trace, and increase the upper trace potential to +0.70 V. We have employed the latter procedure and obtained good agreement in peak height ratios between presumptive MHPG and MHPG standards in duplicate samples (Fig. 2). The drawback of this procedure is that lessening the filter setting while raising the

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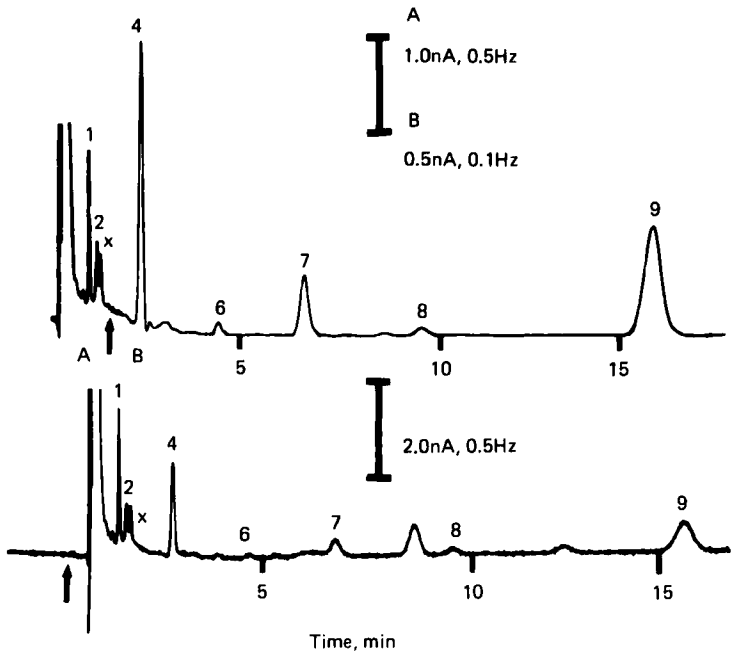

FIGURE 2. Sample dual-parallel chromatogram of spinal cord tissue sonicate using the present method and mobile phase A (see text), illustrating the procedure for obtaining dual potential recording for MHPG. Note the change in sensitivity and filter setting at the arrow in the upper trace. Upper trace recorded at an applied detector potential of +0.70 V versus Ag/AgCl, bottom trace at +0.80 V versus Ag/AgCl. All other conditions including peak identification as in Figure 1.

applied potential raises the detection limit by increasing baseline noise.

Although shorter chromatography times using three micron columns have been reported (2,4), we found that for adequate resolution of complex biological mixtures, it was not feasible to shorten the run time below 15 min, and still resolve early eluting peaks. In fact, Lin et al. (2) reported that they were not able to quantitate MHPG in their system because it was obscured in the

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elution front. While we do not fully take advantage of the "high-speed" aspect of three micron columns, we have realized an average 7-fold increase in sensitivity as compared to five micron columns. Table 2 compares sensitivities obtained on the two column types (same manufacturer) using similar mobile phase systems. This increase in sensitivity when coupled with electronic filtering to lessen baseline noise has resulted in a 10 to 20-fold decrease in detection limits.

The use of dual potential determinations using glassy-carbon electrodes in the dual-parallel mode has allowed us to use direct injections of filtered supernatants with greater confidence of selectivity. We have found that peak height ratios of standards chromatographed at +0.65 V and +0.80 V displayed coefficients of variation of no more than 5%. Thus, this range of variation was chosen as a window for which to evaluate the peak purity of unknowns. It is likely that improvements in detector cell design and/or electrode materials would lower this variation.

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Several performance parameters of the method were evaluated. The intra-assay coefficient of variation was less than 5% for all six compounds of interest and averaged 3.5 $\pm$ 0.5% (N=10). The interassay coefficient of variation, determined from spiked sonicates averaged 5.2 $\pm$ 0.8% for all compounds (N=350). The assay was linear from the detection limit (amount injected) of 5-20 pg to 20 ng for the six analytes. As little as 5 pg NE/mg tissue wet weight to 20 pg HVA/mg tissue wet weight could be quantitated under the conditions employed, where 10% of the total

TABLE 2

Relative sensitivity for analytes of interest using reversed-phase columns packed with 3 micron versus 5 micron particles.

Compound	*Sensitivity 3μm	Sensitivity 5μm
NE	649	127
MHPG	700	128
DA	270	184
HVA	321	15
5-HT	105	12
5-HIAA	343	146

*pA/pmol at +0.65 V versus Ag/AgCl with 0.5 Hz filter in both systems.

tissue content of amines were injected. This was adequate to detect all six compounds in each sample.

The calculated concentrations of the monoamines and metabolites are shown in Table 3. The concentrations of each analyte were not statistically different when animals were sacrificed at 30 min, 60 min, 4 hr and 5 hr after laminectomy. Thus, the data shown in Table 3 is the average ± S.E.M. of all laminectomized animals. In addition, it was found that there were no statistical differences in the concentration of any analyte in adjoining 5 mm segments. Thus, the average values of duplicate determinations in each adjoining segment were averaged.

Using a fluorometric method in the same spinal cord region of the cat, Naftchi et al. (5) reported nearly identical NE levels, two-fold higher 5-HT levels, and nearly ten-fold higher DA levels. These discrepancies may reflect either species differences or the presence of interferences for 5-HT and DA in the less specific batch fluorometric procedure. Using a mass fragmentographic procedure, Commissiong found similar spinal DA levels in the rat to those reported here (6). Similarly, using radioenzymatic

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TABLE 3

Calculated concentrations of spinal cord monoamines and metabolites (μg/g tissue wet weight) in laminectomized rabbits (N=14).

Compound	Spinal Cord Segment*	Concentration ± SEM
5-HT	T_9	0.397 ± 0.045
	T_{10}	0.281 ± 0.023
	T_{11}	0.350 ± 0.036
5-HIAA	T_9	0.091 ± 0.015
	T_{10}	0.079 ± 0.011
	T_{11}	0.114 ± 0.010
NE	T_9	0.178 ± 0.012
	T_{10}	0.155 ± 0.009
	T_{11}	0.156 ± 0.011
MHPG	T_9	0.076 ± 0.011
	T_{10}	0.060 ± 0.010
	T_{11}	0.069 ± 0.010
DA	T_9	0.016 ± 0.001
	T_{10}	0.021 ± 0.004
	T_{11}	0.016 ± 0.002
HVA	T_9	0.038 ± 0.007
	T_{10}	0.030 ± 0.005
	T_{11}	0.041 ± 0.009

*1 cm length of cord corresponding to laminar level noted.

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procedures, Zivin et al. reported similar concentrations of NE, DA and 5-HT in the rabbit spinal cord to those reported here (7).

The utilization ratio, or the ratio of metabolite to putative neurotransmitter, was calculated for each monoamine in order to obtain an index of turnover. These values are shown in Table 4. In agreement with previous findings in the dog brain (8). There was a trend towards higher utilization ratios when the

TABLE 4

Monoamine utilization ratios (metabolite/neurotransmitter) in spinal cord of laminectomized rabbits (N=14).

Compounds	Spinal Cord Segment*	Ratio
5-HIAA/5-HT	T_9	0.173 ± 0.016
	T_{10}	0.194 ± 0.027
	T_{11}	0.157 ± 0.018
MHPG/NE	T_9	0.428 ± 0.055
	T_{10}	0.384 ± 0.059
	T_{11}	0.477 ± 0.090
HVA/DA	T_9	2.310 ± 0.423
	T_{10}	2.083 ± 0.430
	T_{11}	2.121 ± 0.285

*1 cm length of cord corresponding to laminar level noted.

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neurotransmitter concentration was lower, both between and within monoamine systems. These data would indicate that when spinal cord monoamine fibers densely innervate a region, the turnover is lower than when the innervation is sparse. Both NE and 5-HT neurons send dense descending projections to the spinal cord (9,10). There is also literature evidence for descending DA projections (11). The presence of HVA in the spinal cord would indicate that DA is being released from neurons since HVA is an extraneuronal DA metabolite (12). If so, the low concentration of DA and high utilization ratio would indicate that these fibers are sparse and their neurotransmitter is being turnover over rapidly.

In summary, a method for the simultaneous determination of the monoamines and their major metabolites in small segments of spinal cord tissue, with minimal sample handling and pretreatment

is described. The combined use of short reversed-phase columns packed with 3 micron diameter particles and dual-electrode amperometric detection has allowed the resolution and detection of a large number of electroactive species in a relatively short time with a high degree of selectivity.

REFERENCES

1. Kissinger P.T., Brunttett C.S., and Shoup R.E., Neurochemical Applications of Liquid Chromatography with Electrochemical Detection, Life Sci., 28, 455-465, 1981.

2. Lin P.Y.T., Bulawa M.C., Wong P., Lin L., Scott J., and Blank C.L., The Determination of Catecholamines, Indoleamines, Metabolites, and Related Enzymatic Activities Using Three Micron Liquid Chromatography Columns, J. Liq. Chromatogr., 7(3), 509-538, 1984.

3. Mayer, G.S., and Shoup, R.E., Simultaneous Multiple Electrode Liquid Chromatographic-Electrochemical Assay for Catecholamines, Indoleamines, and Metabolites in Brain Tissue, J. Chromatogr., 255, 533-544, 1983.

4. Reinhard Jr., J.F. and Perry J.A. Fast Analysis of Tissue Catechols Using a Short, High Efficiency (3 m) LC Column and Amperometric Detection, J. Liq. Chromatogr. 7(6), 1211-1220, 1984.

5. Naftchi, N.E., Demeny, M., DeCrescito, V., Tomasula, J.J., Flamm, E.S., and Campbell, J.B., Biogenic Amine Concentrations in Traumatized Spinal Cords of Cats: Effect of Drug Therapy, J. Neurosurg., 40, 52-57, 1974.

6. Commissiong, J.W., Synthesis of Catecholamines in the Developing Spinal Cord of the Rat, J. Neurochem., 42, 1574-1581, 1984.

7. Zivin, J.A., Doppman, J.L., Reid, J.L., Tappaz, M.L., Saavedra, J.M., Kopin, I.J., and Jacobowitz, D.M., Biochemical and Histochemical Studies of Biogenic Amines in Spinal Cord Trauma, Neurol., 26, 99-107, 1976.

8. Mefford I.N., Foutz A., Noyee N., Jurik S.M., Handen C., Dement W.C., and Barchas J.D., Distribution of Norepinephrine, Epinephrine, Dopamine, Serotonin, 3,4-Dihydroxyphenylacetic Acid, Homovanillic Acid and 5-Hydroxyindole-3-acetic Acid in Dog Brain, Brain Res., 236, 339-349, 1982.

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9. Tohyama M., Sakai K., Touret M., Salvert D. and Jouvet M., Spinal Projections from the Lower Brainstem in the Cat as Demonstrated by the Horseradish Peroxidase Technique. II. Projections from the Dorsolateral Pontine Tegmentum and Raphi Nuclei, Brain Res., 176, 215-231, 1979.

10. Blessing W.W., Goodchild A.K., Dampney R.A.L., and Chalmers J.P., Cell Groups in the Lower Brainstem of the Rabbit Projecting to the Spinal Cord with Special Reference to Catecholamine-Containing Neurons, Res., 221, 35-55, 1981.

11. Bjorkland, A., and Skagerberg, G., Evidence for a Major Spinal Cord Projection from the Diencephalic A_{11} Dopamine Cell Group in the Rat Using Transmitter-Specific Fluorescent Retrograde Tracing, Brain Res., 177, 170-175, 1979.

12. Cooper J.R., Bloom F.W., Roth R.H., in The Biochemical Basis of Neurophamacology, 4th ed., Oxford University Press, New York, pp. 140-155, 1982.

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