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Evaluating PK/PD Relationship of CNS Drug by Using Liquid Chromatography/ Tandem Mass Spectrometry Coupled to *In Vivo* Microdialysis

Ying Qu^{1*}, Loren Olson², Xiaohui Jiang¹, Leah Aluisio¹, Christopher King¹,
Elliott B. Jones² and Timothy W. Lovenberg¹

¹Johnson & Johnson Pharmaceutical Research & Development,
L. L. C., San Diego, California

²AB SCIEX, Foster City, California
U.S.A.

1. Introduction

Pharmacokinetic (PK) characterization and *in vivo* pharmacological properties of new chemical entities are important components during lead compound selection and optimization in the drug discovery process. Accordingly, reliable techniques are needed that can generate the requisite pharmacokinetic/pharmacodynamic (PK/PD) information for an increased number of compounds. When dealing with compounds targeting the central nervous system (CNS), biophase PK may differ significantly from plasma PK, because blood-brain barrier (BBB) transport and brain distribution often do not occur instantaneously and to a full extent. Therefore the brain distributional behaviors are important determinants of *in vivo* drug effects. *In vivo* microdialysis technique has been used increasingly over the past years for *in vivo* sampling of extracellular exogenous compounds in brains of freely moving rats (1-3). The extracellular brain concentration of free drug which measured by microdialysis may reflect the amount of drug available at the pharmacological target. As the microdialysis technique also allows the simultaneous determination of different endogenous substances, such as neurotransmitters, which sometimes represent the efficacy biomarker of a CNS drug, in the same local interstitial environment. Microdialysis is an attractive tool for PK/PD investigations of CNS active drugs (4). However, the extracellular concentration of neurotransmitters in the synaptic cleft is very low which can be in the range of fg/ μ l; the sampling recovery of microdialysis probe is less than 20%. The application of this technique was highly limited by lack the proper sensitive analytical methods to determine the endogenous substance and exogenous drug.

For analysis of endogenous neurotransmitters, such as, serotonin (5-HT), dopamine (DA), noradrenaline (NE), acetylcholine (ACh), histamine, γ -aminobutyric acid (GABA) and glutamate (Glu), microdialysis in combination with high performance liquid chromatography (HPLC) - electrochemical detection (ED) or fluorescence detection has been

* Corresponding Author

widely used in last two decades (5-6). 5-HT, DA and NE are electrochemically active and can be directly detected in electrochemical detector. However, histamine, Glu and GABA need a pre-column derivatization to produce electrochemical or fluorescence derivatives for their measurement (7-10). The sensitivity of this latter method is further limited by the instability of the final derivatives and the yield of derivatizations. ACh in microdialysate are measured by HPLC-ED following a post-column enzyme reactor and frequently below the detection limit (11). Most investigators have used acetylcholinesterase inhibitors in the perfusion medium to increase basal extracellular ACh concentrations to readily detectable levels (12-13). However, the artificially increased concentration exerts a significant influence on the cholinergic system, thereby making interpretation of drug effects problematic (11, 14-16). For analysis of exogenous drug, HPLC-ED was sensitive, but not applicable since most of drug candidates are electrochemically inactive.

Liquid chromatography/tandem mass spectrometry (LC-MS/MS) technique improvement provides a direct, structural-specific measurement of individual components with very high sensitivity. The mass spectrometer has minimal baseline drift and can be equilibrated very rapidly. However, only a few LC-MS/MS methods for analysis of drugs and individual neurotransmitter in microdialysis sample have been reported (15-19) and analysis all neurotransmitters have never been reported. There are two major problems for the application (20). First, the critical requirement for the use of the LC-MS/MS systems is that the mobile phase must be volatile, because non-volatile ions could significantly reduce the ionization efficiency by ion suppression. The high ionic strength of microdialysis samples generates high background noise and suppresses the ionization of neurotransmitters resulting in considerable reductions in sensitivity and changes in peak shape. Second, these neurotransmitters are polar compounds with low molecular weights; their retentions on standard reversed phase column are generally poor. In the present study, we have used two approaches to solve these problems and developed a series of LC-MS/MS methods which enable us to monitor drug and all neurotransmitters, 5-HT, DA, NE, ACh, Histamine, GABA, Glu, in single microdialysis sample. The methods were applied to study donepezil, a selective acetylcholinesterase inhibitor for treatment of Alzheimer's disease; and citalopram, an antidepressant of selective serotonin reuptake inhibitor. These applications demonstrated *in vivo* microdialysis coupled with LC-MS/MS could 1). Examine the drug efficacy by measuring the changes of extracellular concentration of all neurotransmitters after acute systematic administration of a drug; 2). Estimate the BBB penetration of a drug by measuring the concentration of free drug in the brain interstitial fluid; 3). The time course of free drug in the targeted brain region provided a biophase PK information which was used to evaluate PK/PD relationship by comparing the time course of free drug versus biomarker.

2. Methods

2.1 *In vivo* microdialysis experiments

Male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 300-400 g and were single housed under standard laboratory conditions. Rats were implanted with a probe guide cannula (Eicom, Japan) at the prefrontal cortex (PFC) (21): Incisor bar, -3.5mm, A (anterior to bregma) 3.2 mm, L (lateral from the mid-sagittal suture) 0.8 mm, V (ventral to bregma) 1 mm. Microdialysis experiments were performed at least two days after surgery. Rats were placed in their home cages and connected to a dual channel microdialysis liquid swivel (Instech Solomon, Plymouth Meeting, PA, USA) mounted on a spring loaded counter

balanced arm which allowed animal freely moving. Microdialysis probes have a molecular weight cut-off of 50,000 Da. The exposed tubing of dialysis probe was 4 mm length with an outer diameter of 0.22 mm (Eicom, Japan). The input tube of the dialysis probe was connected to a CMA/102 Microdialysis Pump (CMA, Sweden), which delivered an artificial cerebrospinal fluid (aCSF) containing 147 mM NaCl, 4 mM KCl, 0.85 mM MgCl₂, 2.3 mM CaCl₂. The probe was perfused with aCSF at a rate of 0.6 µl/min overnight to obtain the stable basal level of neurotransmitters. The following morning the flow rate was increased to 1.0 µl/min and microdialysis samples were collected every 60 min to a 96 well plate, starting from 8:00 am for 20 hours. New probes were used every time without determining *in vitro* recovery. Fifteen µl antioxidant which contained 3 mM L-cysteine and 1 mM oxalic acid in 0.1 M acetic acid was added to each sample to prevent serotonin oxidation. Donapazil (2 mg/kg s.c.) or citalopram (10 mg/kg s.c.) were administrated to animal after four baseline microdialysis samples were obtained. The 60 µl microdialysis sample plus 15 µl antioxidant can be aliquot to each 5 µl, then diluted to 50 µl. Ten µl was used for each LC-MS/MS injection. Five to six rats were used for each treatment group. Vehicle animals received 5% N-methyl-2-pyrrolidone solution (v/v, 1 ml/kg, s.c.) injection. The drug concentration in the microdialysis samples were calculated by using *in vitro* recovery of microdialysis probe at a perfusion rate of 1.0 µl/min.

2.2 LC-MS/MS methods

The LC-MS/MS system consisted of two Shimadzu LC-10AD pumps (Shimadzu, Kyoto, Japan) coupled to an Applied Biosystems API-4000™ triple-quadrupole mass spectrometer equipped with a TURBO V™ Ion Source (Applied Biosystems/MDS SCIEX, Toronto, Canada). A CTC Analytics HTS PAL autosampler (Leap Technologies, Carrboro, NC, USA) fitted with a cooled sample tray and a six-port injection valve with a 20 µl injection loop. A ten-port diverter valve was equipped between column and mass spectrometer to divert salt to waste. The mass spectrometer was operated in electrospray positive ion mode and monitored with multiple reaction monitoring (MRM) using the parameters described in Table 1. Data was acquired using Analyst® software (version 1.4.1). The mass spectrometric (ion path) conditions for neurotransmitters and drugs were determined by using the quantitative optimization function of Analyst® software. Different HPLC Methods were developed for each neurotransmitter and drug.

2.2.1 ACh, 5-HT and DA method

100 mm x 2.1 mm i.d., 3 µm, Discovery HS F5 column (Supelco, Bellefonte, PA, USA). Mobile Phase A and B were composed of formic acid (0.1%, v/v) in water and acetonitrile, respectively. An elution profile was composed of an isocratic step of 100% A for 1.5 min and then a linear gradient to 90% B over 3.5 min to separate DA, 5-HT and ACh, kept at 90% B for 1.5 min then equilibrated with 100% A for 1 min. The flow rate was 0.5 ml/min. Valco valve was diverted to MS from 2.5 to 5 min.

2.2.2 Histamine method

100 mm x 2.1 mm i.d., 3 µm, Discovery HS F5 column (Supelco, Bellefonte, PA, USA). Mobile Phase A and B were composed of formic acid (0.05%, v/v) in water and acetonitrile, respectively. An elution profile was composed of an isocratic step of 95% B for 2.0 min and then a linear gradient to 5% B over 0.5 min to let salts elute first, held at 5% B for 4.0 min,

Analyte	DA	5-HT	NA	ACh	Histamine	GABA	Glu	Donepezil	Citalopram
Transition (m/z)	154→137	177→160	170→152	146→60	112→95	104→87	148→84	380→243	325→109
Declustering potential (DP, V)	41	36	31	41	41	21	36	81	76
Collision cell entrance potential (CE, V)	15	16	13	17	21	15	23	37	39
Collision cell exit potential (CXP, V)	10	11	12	4	8	6	6	22	8
Entrance potential (EP)	10	10	10	10	10	10	10	10	10
Collision gas ion energy (CAD)	6	6	5	6	5	5	5	5	5
Curtain gas (CUR)	14	14	15	14	15	15	15	15	15
Ion Source Gas 1 (GS1)	55	55	50	55	50	50	50	50	50
IonSpray Voltage (GS2)	50	50	50	50	50	50	50	50	50
IonSpray Voltage (IS)	3000	3000	3000	3000	3000	3000	3000	3000	3000
Temperature (TEM)	500	500	500	500	650	500	650	600	600

Table 1. The system parameters of mass spectrometry.

then equilibrated with 95% B for 1.5 min. The flow rate was 0.5 ml/min. Valco valve was diverted to MS from 3.8 to 5 min.

2.2.3 Glu method

150 mm x 4 mm i.d., 3 µm, Discovery HS F5 column (Supelco, Bellefonte, PA, USA). Mobile Phase A and B were composed of formic acid (0.05%, v/v) in water and acetonitrile, respectively. An elution profile was composed of an isocratic step of 100% A for 1.5 min and then a linear gradient to 95% B over 0.5 min, held at 95% B for 1.0 min then equilibrated

with 100% A for 3.5 min. The flow rate was 1.2 ml/min. Valco valve was diverted to MS from 1.3 to 2 min.

2.2.4 GABA and NE method

50 mm x 2.1 mm i.d., 5 μ m, 100 Å Primesep 200 column (SIELC Technologies, Prospect Heights, IL, USA). Mobile Phase A and B were composed of acetic acid (0.2%, v/v) in water and acetonitrile, respectively. An elution profile composed of an isocratic step of 90% A for 1.5 min and then a linear gradient to 90% B over 3.5 min for separation, hold at 90% B for 1.5 min then equilibrate with 100% A for 1 min. The flow rate was 0.5 ml/min. Valco valve was diverted to MS from 2.5 to 5 min.

2.2.5 Donepezil and citalopram method

30 mm x 2.0 mm i.d., Synergi 4 μ Hydro-RP 80 Å column (Phenomenex, Torrance, CA, USA). Mobile Phase A and B were composed of formic acid (0.05%, v/v) in water and acetonitrile, respectively. An elution profile was composed of an isocratic step of 90% A for 1.5 min and then a linear gradient to 90% B over 3.5 min for separation, held at 90% B for 1.5 min then equilibrated with 100% A for 1 min. The flow rate was 0.5 ml/min. Valco valve was diverted to MS from 2.5 to 5 min.

2.3 Analysis of extracellular 5-HT and NE levels by HPLC-ED

Compared to LC-MS/MS method, 5-HT and NE are easier to measure by high-performance liquid chromatography (ESA Model 582) coupled to an electrochemical detector (CoulArray Coulometric, ESA) with dual channel coulometric microdialysis cell (ESA 5014B). Separation was performed on a C18 column (Hypersil, 150 x 3.2 mm I.D.) at room temperature. The mobile phase consisted of 75 mM NaH₂PO₄, 0.5 mM Disodium-EDTA, 350 mg/L 1-octanesulfonic acid, pH 3.1, 1.0% THF, 9.0% ACN. The flow rate was 0.4 ml/min. 22 μ l microdialysate was injected by an autosampler (ESA Model 540). The first electrode of the detector was set at -90 mV (reduction) and the second at +280 mV (oxidation).

2.4 Statistical analysis

All values for microdialysis studies were calculated as percentage change at each time point compared with the average of four baseline values. The extracellular neurotransmitter concentration at each time point after vehicle injection was compared with the average of four baseline values by one way ANOVA - Dunnett's test for their significant differences.

The overall effect of drug treatments on extracellular neurotransmitter levels was determined by a two way ANOVA with treatment as the independent variable and time as the repeated measurement. If significant, the ANOVA was followed by post-hoc Duncan's multiple range test (SigmaStat, SPSS Inc., www.spss.com).

3. Results

Figure 1 shows the mass spectra and molecular structures of precursor ions and associated daughter ions of neurotransmitters, donepezil and citalopram. The spectra show several prominent fragment ions. The ion that was selected for the detection of each neurotransmitter and drug was always the highest intensity ion except ACh. For ACh the lower intensity ion at $m/z = 60$ gave greater signal to noise than the higher intensity

product ion at $m/z = 86$. A summary of MRM parameters for all positive mode analytes was given in table 1.

Figure 2 shows the representative chromatogram of each neurotransmitter and drug in standard mixture solution (1 pg/ μ l standard in 1:10 dilution of aCSF) and in 1:10 dilution of basal level microdialysates from frontal cortex of freely moving rat.

3.1 Drug effect on the extracellular neurotransmitters levels in rat frontal cortex

The LC-MS/MS methods were applied to evaluate the effects of acute administration of either donepezil or citalopram on the extracellular concentration of ACh, Histamine, GABA, Glu, DA, NE and 5-HT in rat frontal cortex (as shown in Figure 3). Before dosing the animal, four 60 μ l microdialysis samples were collected and the basal extracellular concentration of neurotransmitters in rat frontal cortex which were measured by LC-MS/MS methods are (mean \pm S.E.): ACh, 1.93 ± 0.14 pg/ μ l (n=15); Histamine, 1.2 ± 0.15 pg/ μ l (n=15); GABA, 6.25 ± 0.35 pg/ μ l (n=15); Glu, 2.44 ± 0.25 ng/ μ l (n=15); DA, 0.15 ± 0.008 pg/ μ l (n=15); NE, 0.40 ± 0.015 pg/ μ l (n=8) and 5-HT, 0.20 ± 0.016 pg/ μ l (n=6). Since the LC-MS/MS methods presented here required a 10-fold dilution of the microdialysis sample, the 5-HT and NE detection limit was challenging and the HPLC-ED was a preferred method for these analytes. The basal extracellular concentrations of 5-HT and NE (Mean \pm S.E.) in frontal cortex which measured by HPLC-ED were NE: 0.36 ± 0.15 pg/ml (n=20) and 5-HT, 0.10 ± 0.06 pg/ μ l (n=50).

Vehicle administration (n=5) did not significantly alter basal extracellular ACh, Histamine, GABA, Glu, DA, NE and 5-HT concentration in rat frontal cortex as shown in Figure 3. The acute administration of donepezil (2.0 mg/kg, s.c., n=5) evoked a significant increase of extracellular ACh from basal levels in the frontal cortex of rats compared with vehicle animal, to a maximum of $645 \pm 69\%$ at the first hours post dose and the average over the 3-6 hour treatment period was sustained at $178 \pm 12\%$ of basal values (as shown in Figure 3A). Donepezil did not significantly change the extracellular concentration of Histamine, GABA, DA, NE and 5-HT (Figure 3B, 3C, 3E, 3F and 3G), but significantly decreased the Glu level in frontal cortex of rats compared with vehicle animal. Glu concentration continually decreased to a maximum of $57 \pm 17\%$ at the sixth hour post dose (Figure 3D).

The acute administration of citalopram (10 mg/kg, s.c., n=5) immediately evoked a significant increase of extracellular 5-HT from basal levels in the frontal cortex of rats compared to vehicle animals at the first hour post dose and the average over the 6 hour treatment period was sustained at $412 \pm 14\%$ of basal values (as shown in Figure 3G). Citalopram did not significantly change the extracellular concentration of ACh, Histamine, Glu, DA and NE, (Figure 3A, 3B, 3D, 3E and 3F), but significantly decreased GABA level in frontal cortex of rats compared with vehicle animal. GABA level decreased to a maximum of $53 \pm 9.4\%$ at the forth hour post dose and the average over the 2-6 hour treatment period was sustained at $69\% \pm 4.6\%$ of basal values (as shown in Figure 3C).

3.2 Biophase PK profile and the PK/PD relationship

The biophase PK profiles of donepezil and citalopram (as shown in Figure 4) were generated by measuring the extracellular concentration of free donepezil or citalopram in midialysate of rat frontal cortex using LC-MS/MS methods. The PK profile was compared with the time course of extracellular concentration of neurotransmitters in the same microdialysate of frontal cortex to evaluate their PK/PD relationship (as shown in Figure 4).

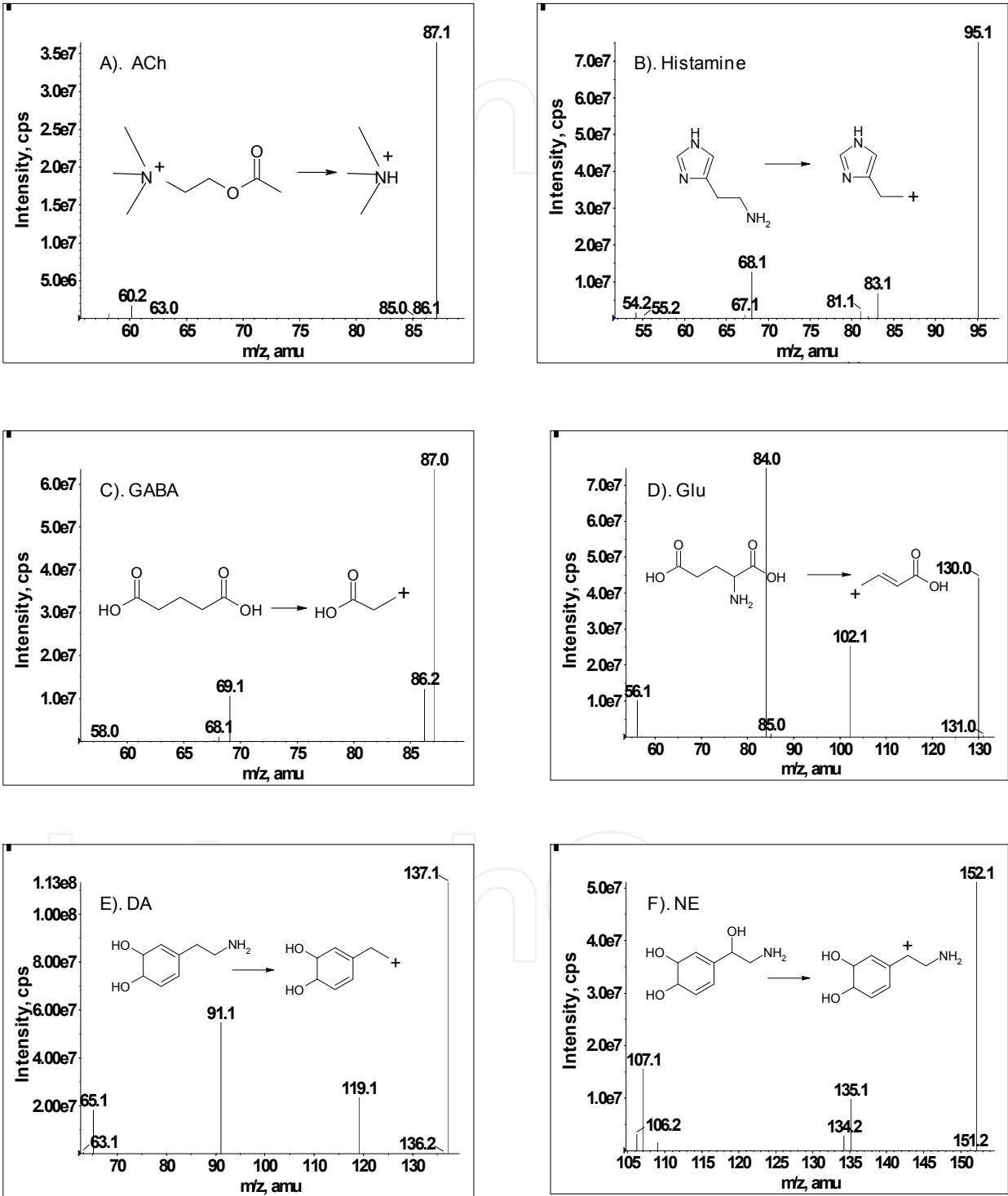
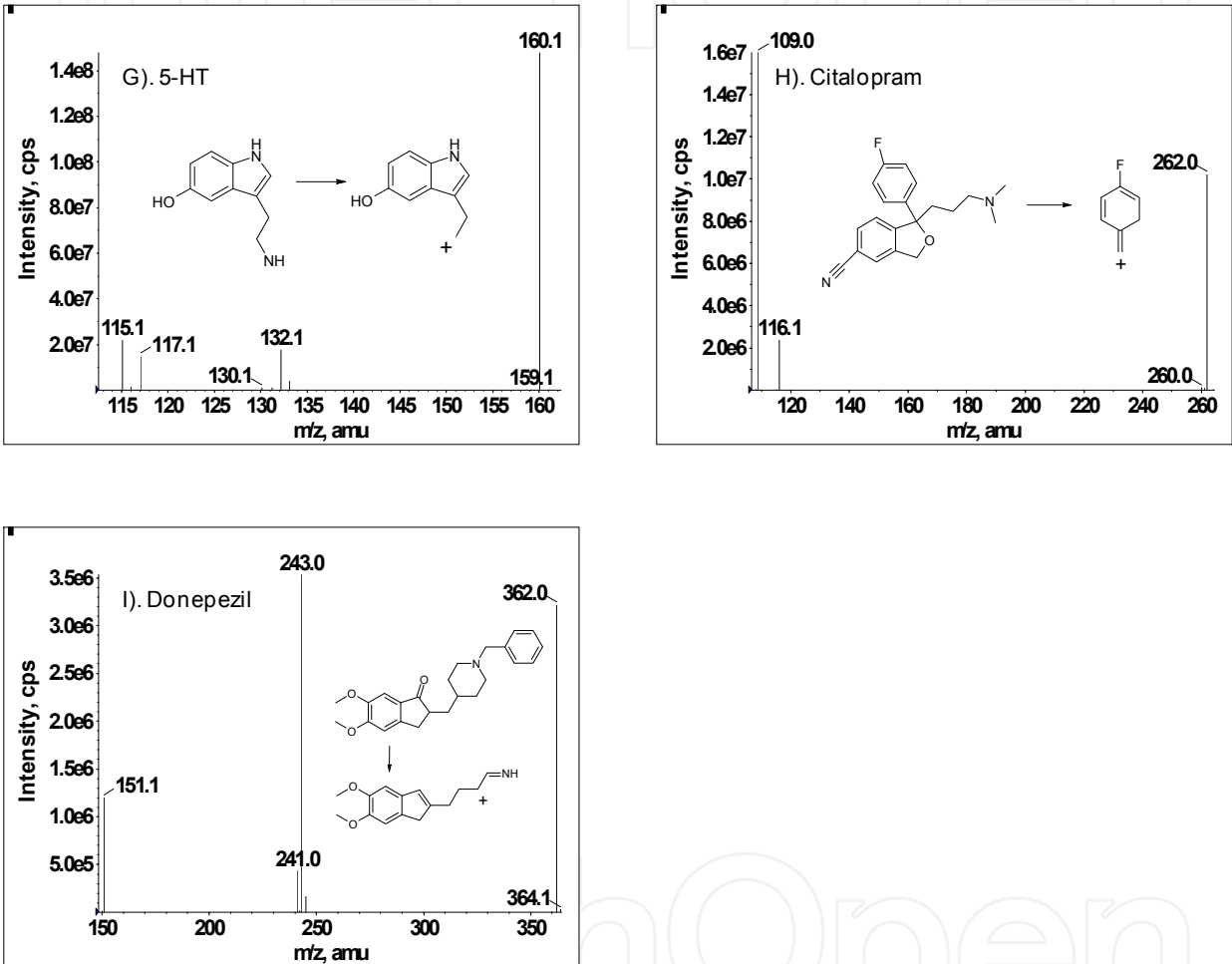


Fig. 1. Part I



Part II

Fig. 1. Mass spectra and molecular structures of precursor ions and associated daughter ions for A). ACh; B). Histamine; C). GABA; D). Glu; E). DA; F). NE; G). 5-HT; H). citalopram; I). donepezil.

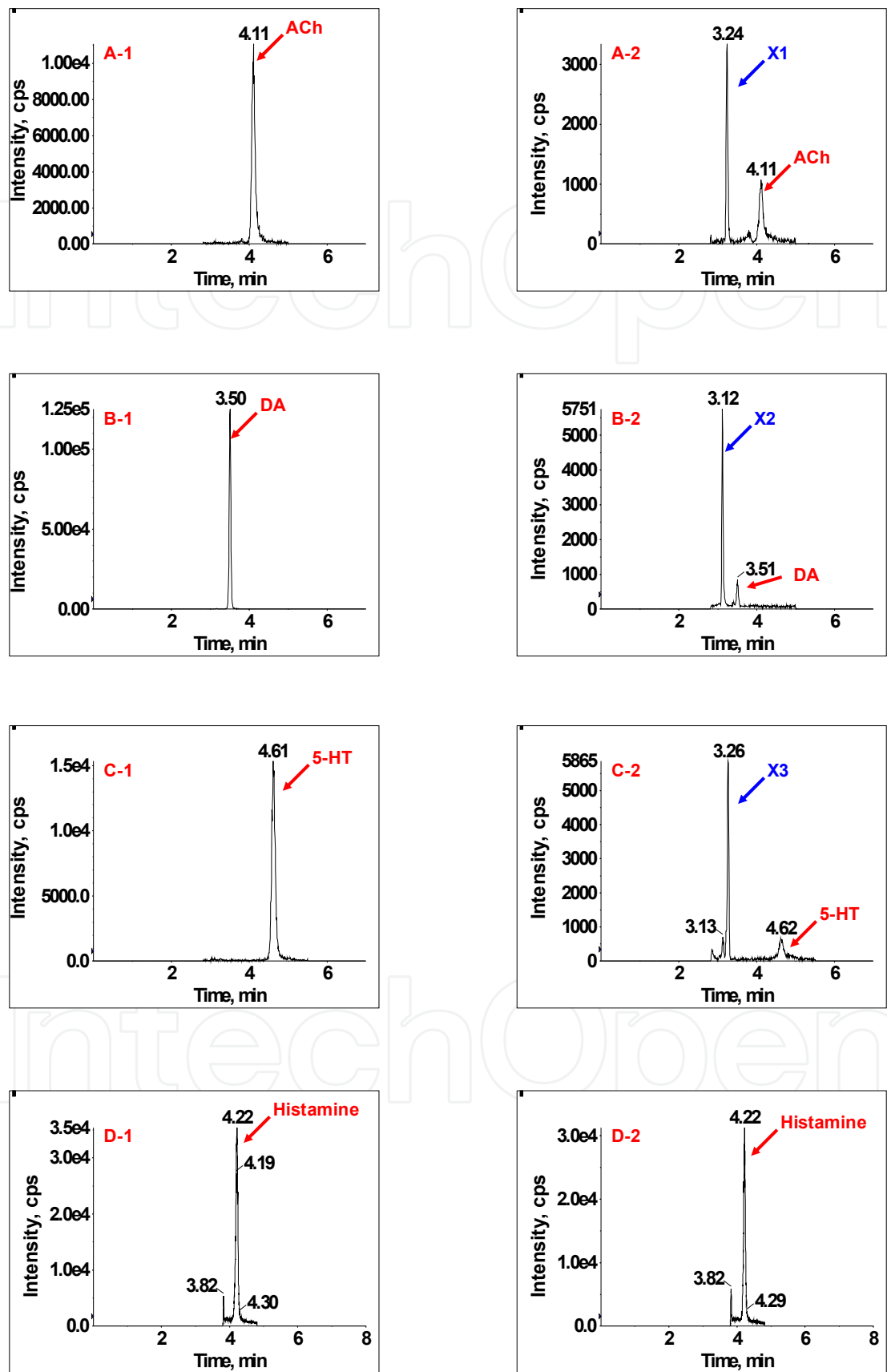


Fig. 2. Part I

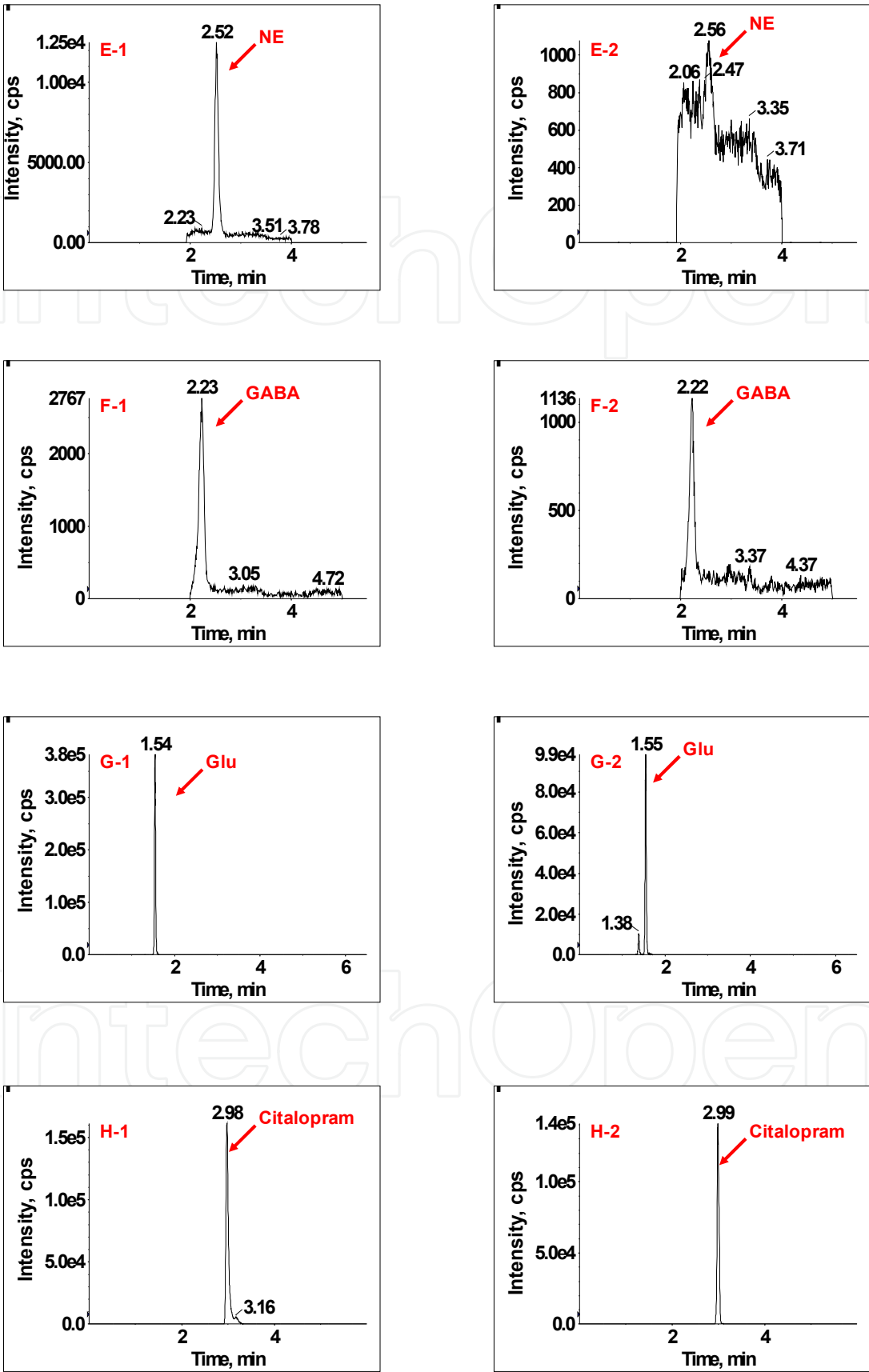
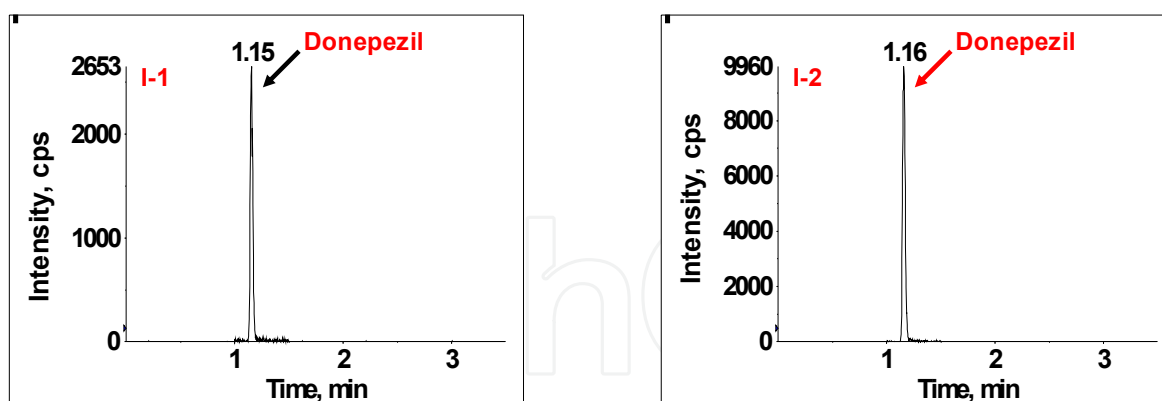


Fig. 2. Part II



Part III

Fig. 2. The representative chromatograms of a 1 pg/ μ l standard mixture of neurotransmitters and drugs in 1:10 dilution of aCSF. A-1. ACh; B-1. DA; C-1. 5-HT; D-1. Histamine; E-1. NE; F-1. GABA; G-1. Glu; H-1. Citalopram; I-1. Donepezil. The representative chromatograms of 1:10 dilution of basal level microdialysates from frontal cortex of freely moving rat for each neurotransmitter and drug at the first hour after drug administration: A-2. ACh; B-2. DA; C-2. 5-HT; D-2. Histamine; E-2. NE; F-2. GABA; G-2. Glu; H-2. Citalopram; I-2. Donepezil. X1: the unknown molecule share the same transition with ACh (m/z 146 \rightarrow 87); X2: the unknown molecule share the same transition with DA (m/z 154 \rightarrow 137); X3: the unknown molecule share the same transition with 5-HT (m/z 177 \rightarrow 160).

Donepezil is a centrally acting reversible acetylcholinesterase inhibitor. Its main therapeutic use is in the treatment of Alzheimer's disease where it is used to increase cortical ACh release and modest benefits in cognition and/or behavior. Therefore the extracellular ACh level is the efficacy biomarker for donepezil. Figure 4A shows the comparison of time course of donepezil and its effect on ACh efflux in frontal cortex after donepezil (2 mg/kg, s.c.) administration. The immediate increase in donepezil concentrations was associated directly with a concomitant increase in extracellular ACh levels within the first hour, which closely followed the fast clearance of donepezil from extracellular fluid. The donepezil concentration in microdialysate increased to the peak concentration, 157 ± 23 ng/ml and gradually decreased to minimum concentration 5.5 ± 0.51 ng/ml at end of experiment (18 hours after administration).

Citalopram is an antidepressant used to treat major depression associated with mood disorders. Citalopram belongs to selective serotonin reuptake inhibitors, which exert their therapeutic effects by increasing extracellular 5-HT level in the synaptic cleft. Figure 4B shows the comparison of time course of citalopram concentration and its effect on 5-HT efflux in frontal cortex after citalopram (10 mg/kg, s.c.) administration. Interestingly, the immediate increase in citalopram concentrations was associated directly with an increase in extracellular 5-HT levels, however 5-HT level did not follow the fast clearance of citalopram from extracellular fluid and kept increasing to the peak release ($545 \pm 50\%$ of basal level) at 9 hours after dosing, while remaining elevated for at least 18 hours. The citalopram concentration in microdialysate increased to the peak concentration, 281 ± 37 ng/ml within 1 hour of administration and was eliminated within 17 hours. The persistent increase in 5-HT concentrations may be related to the effect of citalopram metabolite on 5-HT release.

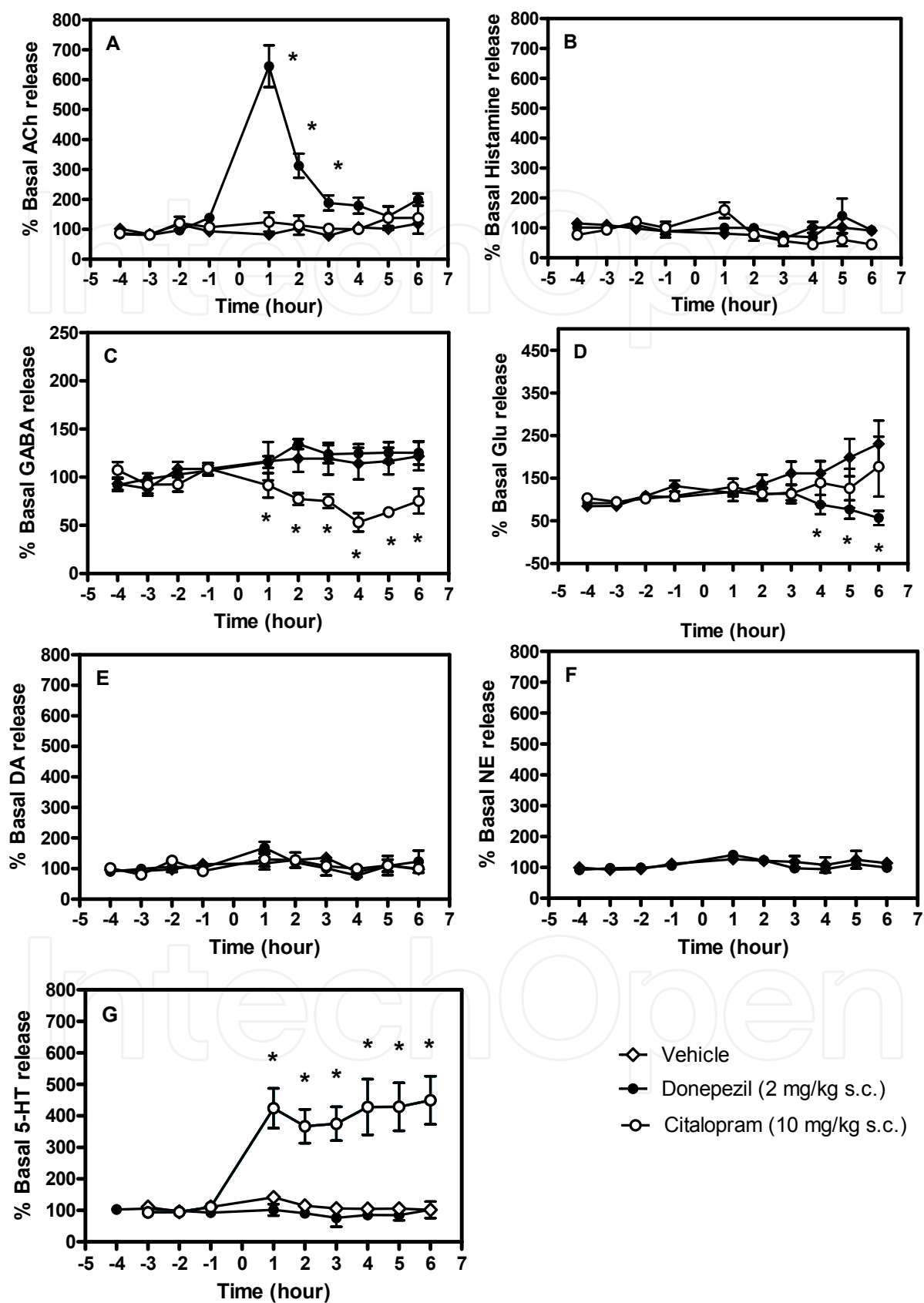


Fig. 3. Effect of donepezil (2 mg/kg, s.c., n=5) and citalopram (10 mg/kg, s.c., n=5) on extracellular concentration of neurotransmitters in the frontal cortex of rat: A. ACh; B.

Histamine; C. GABA; D. Glu; E. DA; F. NE and G. 5-HT. Values are mean \pm S.E.M.. The neurotransmitter levels expressed as a percentage of the average of three baseline samples (defined as 100%). Two ways ANOVA - post-hoc Duncan's multiple range test were used for comparison. (Asterisks indicate significance of overall effect of drug treatment versus vehicle: * $P<0.05$, ** $P<0.01$, *** $P<0.001$.)

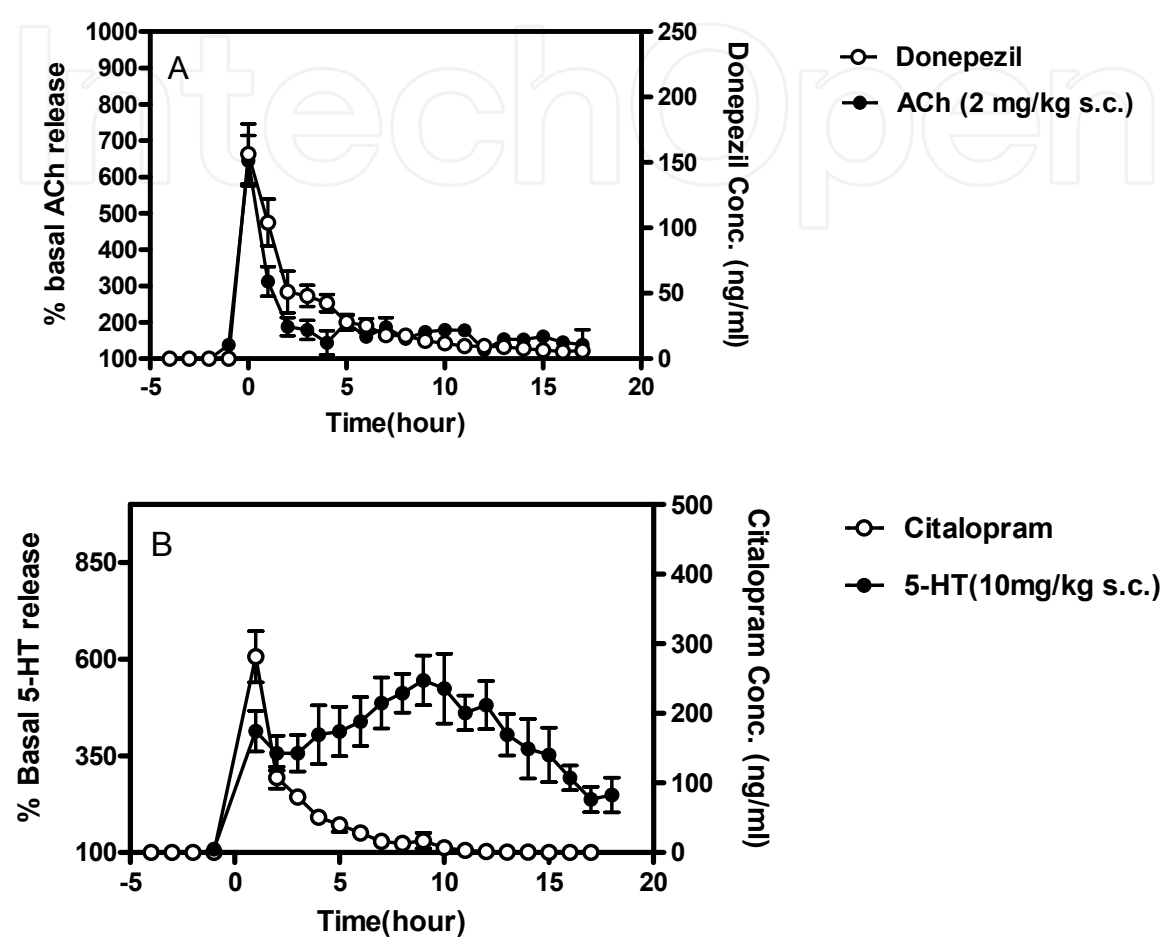


Fig. 4. PK/PD profiles of Donepezil and Citalopram: A. The extracellular concentration of donepezil and ACh in the frontal cortex of rats after Donepezil (2 mg/kg, s.c.) administration; B. The extracellular concentration of citalopram and 5-HT in the frontal cortex of rats after citalopram (10 mg/kg, s.c.) administration. Values are mean \pm S.E.M. The neurotransmitter levels was expressed as a percentage of the average of three baseline samples (defined as 100%) and the drug concentration was expressed as ng/ml.

4. Discussion

In these studies, *in vivo* microdialysis in conjunction with automated sampling for 24 hours in freely moving rats provided a very useful technique for collecting the endogenous neurochemicals and exogenous drugs in the specific brain region without stress from sampling procedures or anesthesia. Analysis of these samples with LC-MS/MS methods, which provide high sensitivity and structural specificity, make it possible to monitor all neurotransmitter release in single sample collection and is a valuable tool for evaluating PK/PD relationship of new chemical entities in drug discovery.

LC-MS/MS applications in measuring neurotransmitters in microdialysis samples were limited by poor retention on reversed column and high salted aCSF matrix (20). To solve the first problem, two novel stationary phase columns were used in this study. One column is a Discovery F5 column, which uses pentafluorophenyl (PEP)(22) as a function group to attach on the silica via a propyl chain. The PEP type phase provides greater retention of polar solutes without ion-pairing or ion-suppressing agents in the mobile phase. Another column is Primesep 200 (23), a reverse-phase analytical column with embedded weak acidic ion-pairing groups. It improves retention of strong basic compounds by cation-exchange mechanism and retains neutral compounds by reverse-phase mechanism. All mobile phases for these two columns were LC-MS mobile phase (acetonitrile with formic acid or acetic acid). ACh, DA, 5-HT, histamine, NE, GABA and Glu were retained in these columns for 2-5 min, which is sufficient to separate them from high salt and endogenous interferences in microdialysis sample. The effluent that contained salts and the major ion-suppressing endogenous interferences were diverted to waste for the first 2-3 min as the online desalt step. In addition, the microdialysis sample (60 μ l in each collection) was diluted to 1:10 by water to further reduce ion-suppressing influence from salt and endogenous interferences. 600 μ l sample volume could make several LC-MS/MS injections for monitoring different biomarkers or drug in different methods, since the typical LC injection volume is 10 μ l.

In contrast to the neurotransmitters, the average molecular weight of drug was 300-600 and drug molecule is very easy to retain on reverse phase column and separated with salt and ion-suppression agents. Therefore reverse phase column was used for drug LC-MS/MS method. We were unable to improve the 5-HT and NE sensitivity for LC-MS/MS method to monitor them at a 1 to 10 dilution of microdialysate. As discussed before, they have very good electrochemical activity and therefore the best way to monitor 5-HT and NE is still HPLC-ED.

The mass spectrometry conditions for each neurotransmitter and drug in this study are similar with other studies (15-19). In addition, compared with the chromatogram of neurotransmitter's standard solution (as shown in Figure 2), some unknown peaks in microdialysis were observed by LC-MS/MS. These unknown peaks always had the same transition (m/z) as the neurotransmitters, as shown in Figure 2. ACh (m/z 146 \rightarrow 87) was eluted at 4.11 min and peak X1 (m/z 146 \rightarrow 87) was eluted at 3.24 min (Figure 2A-2). DA (m/z 154 \rightarrow 137) was eluted at 3.51 min and peak X2 (m/z 154 \rightarrow 137) at 3.12 min (Figure 2B-2); 5-HT (m/z 177 \rightarrow 160) was eluted at 4.62 min and peak X3 (m/z 177 \rightarrow 160) at 3.26 min (Figure 2C-2). A similar phenomenon has been observed in a study by Zhu et al. (17). In their full scan MS/MS experiments, the (3-carboxypropyl) trimethylammonium was identified, which was the unknown peak and had the same transition as ACh (m/z 146 \rightarrow 87). (3-carboxypropyl) trimethylammonium has been reported to be a substrate in the production of γ -betaine hydroxylase, an enzyme in the biosynthesis of carnithine. A further study to identify these unknown peaks will be very interesting and may lead us to find new endogenous substance in the brain and expand our knowledge of neurochemistry.

In this study, for the first time we report the effect of citalopram (and donepezil) on seven neurotransmitter release. In agreement with literatures, 10 mg/kg citalopram in this study preferentially elevated levels of 5-HT in the frontal cortex of freely moving rats (24-25). DA and NE were not changed by citalopram at this dose; this agrees with Millan et al.'s results (26). It only evoked a slight rise in levels of NE and DA even at the highest dose (40 mg/kg) (26). Interestingly 10 mg/kg citalopram also markedly decreased extracellular GABA

concentration in the frontal cortex in the same animals. To our knowledge, others have not reported this result, which is a favorable effect for depression patients. 10 mg/kg citalopram did not change the extracellular concentration of ACh, histamine and Glu in the present study.

Donepezil, a potent and selective acetylcholinesterase inhibitor for the treatment of Alzheimer's disease. Our results show that subcutaneous injection of donepezil (2 mg/kg) produced a marked (up to 7-fold) elevation of extracellular ACh in the frontal cortex. In previously studies, Giacobini et al. (27) examined the effect of subcutaneous injection of donepezil (0.5 and 2 mg/kg) on extracellular ACh concentration in the cerebral cortex of rats by using transcortical microdialysis. In their experimental, 2 mg/kg donepezil produced 20-fold elevation of extracellular ACh in the cortex. It had the similar time to the maximum plasma concentration (1 hour after dosing) and duration (3-6 hours) (28). Our study is the first to report changes in extracellular Glu cortical levels in parallel with ACh after donepezil administration. A similar result has been reported: ENA713 (29), a novel acetylcholinesterase inhibitor, significantly decreased extracellular glutamate level in rat hippocampus by using *in vivo* microdialysis. Decreased Glu was a favorable effect for treatment of Alzheimer's disease, since increased cerebrospinal fluid Glu concentration has been reported in Alzheimer's disease patients (30). These applications provided a very good example to screen CNS drug by comparing their drug effects on multiple neurotransmitters release with LC-MS/MS coupled to *in vivo* microdialysis. The results may help us to find the new therapeutic indication for an old drug.

In present study, the basal level 5-HT, NE and DA in the frontal cortex of rats which were measured by LC-MS/MS were 0.20 ± 0.016 pg/ μ l (n=6), 0.40 ± 0.015 pg/ μ l (n=8), 0.15 ± 0.008 pg/ μ l respectively, These results are correlated with the results in our lab that measured by HPLC-ED: 0.10 ± 0.06 pg/ μ l (n=50) for 5-HT; 0.36 ± 0.15 pg/ μ l (n=20) for NE and 0.06 ± 0.05 pg/ μ l (n=20) for DA. Due to a different route of administration and possible difference in the microdialysis techniques, the basal levels of other neurotransmitters in the frontal cortex of rats were slightly different in our study than in others, but were in similar ranges.

Since the method development of LC-MS/MS for drugs is relatively easier than for neurotransmitters and is amenable to a 1/10 dilution of microdialysis samples, this provides a unique opportunity to estimate drug CNS penetration and elucidate PK/PD profiles by monitoring concentrations of a drug simultaneous with drug induced neurotransmitter release in the same animal. In Figure 4A, administration of 2 mg/kg donepezil caused a rapid increase in ACh. The extracellular donepezil concentration in the frontal cortex of rats was associated with a concomitant increased in extracellular ACh level and reached to the maximal concentration in the same time. The time-course of donepezil induced ACh efflux, which represents drug's efficacy or PD property, correlated well with the concentration curve of donepezil. This result suggested that donepezil had very fast blood-brain barrier penetration and inhibited the ChE, which directly elevated ACh release on the site of drug action. This result demonstrates good agreement with drug PK/PD properties.

In Figure 4B, administration of 10 mg/kg citalopram caused an immediate increase of 5-HT in rat frontal cortex. However, the time-course of citalopram induced 5-HT efflux did not parallel the biophase PK profile of citalopram. This finding indicates that the time-course for extracellular 5-HT in the frontal cortex reflects a more complex mechanism than direct action of citalopram on 5-HT reuptake inhibition at its dose. Citalopram has several

metabolites, demethylcitalopram (DCIT), didemethylcitalopram (DDCIT), and citalopram N-oxide (CIT-NO) in human plasma and urine (31-32). Due to lack of commercial standards of citalopram metabolite, their concentration in microdialysate could not be measured in this study.

These examples illustrate the applicability of the LC-MS/MS coupled to *in vivo* microdialysis technique to rapidly estimate the PK/PD profiles of novel substances. This technique will be a very useful tool to study the drug penetration of BBB in lead optimization of drug discovery. Compared to the traditional method for the study of drug BBB penetration (33), this technique avoids having to euthanize the animal at each time point to measure drug concentration in whole brain tissue and provides continually monitoring extracellular drug concentration in specific brain regions at each hour for a 24-hour period by using a single animal as shown in table 2.

	Traditional Method	<i>In vivo</i> microdialysis-LC/MS/MS method
Specificity	<ul style="list-style-type: none">• Using whole brain tissue• Measure total (intracellular plus extracellular) drug concentration	<ul style="list-style-type: none">• Sample from individual brain region (such as frontal cortex, striatum, hippocampus, hypothalamus)• Measure extracellular drug concentration
Efficiency	<ul style="list-style-type: none">• Using 8 animals to get 8 time points• More labor to sacrifice each animal, homogenate brain• Sample preparation for LC injection	<ul style="list-style-type: none">• Using 1 animal to get 24 time points• Automatic sample collection for 24 hours without supervision. Only need to implant microdialysis probe guide by brain surgery.• Sample ready for LC injection
Information	<ul style="list-style-type: none">• PK data (drug concentration in the brain)	<ul style="list-style-type: none">• PK and PD data (both drug and biomarker concentration)

Table 2. Comparison of drug blood-brain barrier (BBB) penetration study by using traditional method versus *in vivo* microdialysis-LC/MS/MS method.

5. Conclusion

This study demonstrated that liquid Chromatography/tandem mass spectrometry (LC-MS/MS) coupled with *in vivo* microdialysis provides a powerful method for the measurement of endogenous and exogenous substances in the brain interstitial fluid (ISF) surrounding the probe and so it represents an important tool in CNS drug discovery. 1). It can be used to measure the pharmacodynamic response of neuroactive compounds, represents the **drug efficacy**, by measuring neurotransmitters and other biomarkers; 2). A

core requirement for an effective CNS drug is an ability to cross the BBB and remain in the brain ISF for sufficient duration and concentration to evoke the desired therapeutic effect. LC-MS/MS coupled with *in vivo* microdialysis can be used to evaluate the **BBB penetration** of drug candidate by measuring the free concentration of CNS drug in brain ISF; 3). The profile of time-free concentration of CNS drug in the targeted brain region provides **biophase pharmacokinetic** information which can differ significantly from pharmacokinetics in plasma. Establishing **PK-PD relationship** of the drug, especially biophase PK, allow better understanding of exposure-response relationships and help the selection of drug; 4). In some case, not parent drug, but **drug metabolite** contributes to efficacy or toxicity. LC-MS/MS coupled with *in vivo* microdialysis also can be used to study drug metabolism during drug discovery.

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7. References

- Alavijeh, M., and Palmer, A. (2010) Measurement of the pharmacokinetics and pharmacodynamics of neuroactive compounds, *Neurobiol Dis.* 37, 38-47.
- Barbier, A., Aluisio, L., Lord, B., Qu, Y., Wilson, S., Boggs, J., Bonaventure, P., Miller, K., Fraser, I., Dvorak, L., Pudiak, C., Dugovic, C., Shelton, J., Mazur, C., Letavic, M., Carruthers, N., and Lovenberg, T. (2007) Barbier, A.J. et al. Pharmacological characterization of JNJ-28583867, a histamine H(3) receptor antagonist and serotonin reuptake inhibitor *Eur J Pharmacol* 576, 43-54
- Berzas-Nevado, J., Villasenor-Llerena, M., Guiberteau-Cabanillas, C., and Rodriguez-Robledo, V. (2006) Enantiomeric screening of racemic citalopram and metabolites in human urine by entangled polymer solution capillary electrophoresis: an innovatory robustness/ruggedness study *Electrophoresis* 27, 905-917.
- Blakeley, J., and Portnow, J. (2010) Microdialysis for assessing intratumoral drug disposition in brain cancers: a tool for rational drug development, *Expert Opin Drug Metab Toxicol.* 6, 1477-1491.
- Ceglia, I., Carlik, M., Baviera, M., Renoldi, G., Calcagno, E., and Invernizzi, R. (2004) The 5-HT receptor antagonist M100,907 prevents extracellular glutamate rising in response to NMDA receptor blockade in the mPFC. , *J Neurochem* 91, 189-199.
- Chu, M., Huang, Z., Qu, W., Eguchi, N., Yao, M., and Urade, Y. (2004) Chu, M. et al. Extracellular histamine level in the frontal cortex is positively correlated with the amount of wakefulness in rats, *Neurosci Res* 49, 417-420.
- Csernansky, J., Bardgett, M., Sheline, Y., Morris, J., and Olney, J. (1996) CSF excitatory amino acids and severity of illness in Alzheimer's disease *Neurology* 46, 1715-1720.

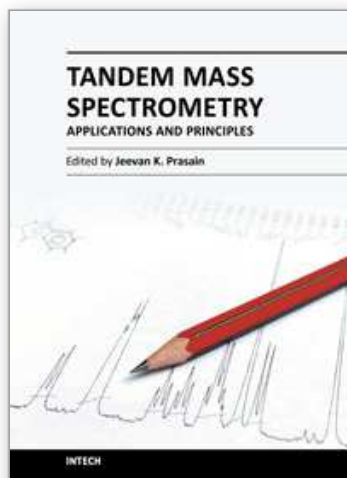
- David, D., Bourin, M., Jegu, G., Przybylski, C., Jolliet, P., and Gardier, A. (2003) David, D.J. et al. Effects of acute treatment with paroxetine, citalopram and venlafaxine in vivo on noradrenaline and serotonin outflow: a microdialysis study in Swiss mice, *Br J Pharmacol* 140, 1128-1136.
- de Lange, E., Ravenstijn, P., Groenendaal, D., and van Steeg, T. (2005) Toward the prediction of CNS drug-effect profiles in physiological and pathological conditions using microdialysis and mechanism-based pharmacokinetic-pharmacodynamic modeling, *AAPS J* 7, E532-543.
- Del Arco, A., and Mora, F. (2000) Endogenous dopamine potentiates the effects of glutamate on extracellular GABA in the prefrontal cortex of the freely moving rat, *Brain Res Bull* 53, 339-345.
- Felton, T., Kang, T., Hjorth, S., and Auerbach, S. (2003) Effects of selective serotonin and serotonin/noradrenaline reuptake inhibitors on extracellular serotonin in rat diencephalon and frontal cortex, *Naunyn Schmiedeberg's Arch Pharmacol* 367, 297-305.
- Giacobini, E., Zhu, X., Williams, E., and Sherman, K. (1996) The effect of the selective reversible acetylcholinesterase inhibitor E2020 on extracellular acetylcholine and biogenic amine levels in rat cortex *Neuropharmacology* 35, 205-211.
- Hows, M., Lacroix, L., Heidbreder, C., Organ, A., and Shah, A. (2004) High-performance liquid chromatography/tandem mass spectrometric assay for the simultaneous measurement of dopamine, norepinephrine, 5-hydroxytryptamine and cocaine in biological samples *J Neurosci Methods* 138, 123-132.
- Hows, M., Organ, A., Murray, S., Dawson, L., Foxton, R., Heidbreder, C., Hughes, Z., Lacroix, L., and Shah, A. (2002) High-performance liquid chromatography/tandem mass spectrometry assay for the rapid high sensitivity measurement of basal acetylcholine from microdialysates *J Neurosci Methods* 121, 33-39.
- Ichikawa, J., Dai, J., and Meltzer, H. (2000) Acetylcholinesterase Inhibitors are neither necessary nor desirable for microdialysis studies of brain acetylcholine *Current Separation* 19, 37-43.
- Ichikawa, J., Dai, J., O'Laughlin, I., Fowler, W., and Meltzer, H. (2002) Atypical, but not typical, antipsychotic drugs increase cortical acetylcholine release without an effect in the nucleus accumbens or striatum *Neuropsychopharmacology* 26, 325-339.
- Kawashima, K., Sato, A., Yoshizawa, M., Fujii, T., Fujimoto, K., and Suzuki, T. (1994) Effects of the centrally acting cholinesterase inhibitors tetrahydroaminoacridine and E2020 on the basal concentration of extracellular acetylcholine in the hippocampus of freely moving rats *Naunyn Schmiedeberg's Arch Pharmacol* 350, 523-528.
- Keski-Rahkonen, P., Lehtonen, M., Ihalainen, J., Sarajarvi, T., and Auriola, S. (2007) Quantitative determination of acetylcholine in microdialysis samples using liquid chromatography/atmospheric pressure spray ionization mass spectrometry *Rapid Commun Mass Spectrom* 21, 2933-2943.
- Kissinger, P. (1989) Biomedical applications of liquid chromatography-electrochemistry, *J Chromatogr.* 488, 31-52.

- Kosasa, T., Kuriya, Y., and Yamanishi, Y. (1999) Effect of donepezil hydrochloride (E2020) on extracellular acetylcholine concentration in the cerebral cortex of rats *Jpn J Pharmacol* 81, 216-222.
- Lacroix, L., Hows, M., Shah, A., Hagan, J., and Heidbreder, C. (2003) Selective antagonism at dopamine D3 receptors enhances monoaminergic and cholinergic neurotransmission in the rat anterior cingulate cortex *Neuropsychopharmacology* 28, 839-849.
- Li, J., Shao, S., Jaworsky, M., and Kurtulik, P. (2008) Simultaneous determination of cations, zwitterions and neutral compounds using mixed-mode reversed-phase and cation-exchange high-performance liquid chromatography *J Chromatogr A* 1185, 185-193.
- Maura, G., and Raiteri, M. (1986) Cholinergic terminals in rat hippocampus possess 5-HT1B receptors mediating inhibition of acetylcholine release, *Eur J Pharmacol* 129, 333-337.
- Millan, M., Dekeyne, A., Papp, M., La Rochelle, C., MacSweeney, C., Peglion, J., and Brocco, M. (2001) S33005, a novel ligand at both serotonin and norepinephrine transporters: II. Behavioral profile in comparison with venlafaxine, reboxetine, citalopram, and clomipramine, *J Pharmacol Exp Ther* 298, 581-591.
- Needham, S., Brown, P., and Duff, K. (1999) Phenyl ring structures as stationary phases for the high performance liquid chromatography electrospray ionization mass spectrometric analysis of basic pharmaceuticals, *Rapid Commun Mass Spectrom* 13, 2231-2236.
- Paxinos, G., and Watson, C. (1997) *The Rat Brain in Stereotaxic Coordinates*, Academic Press San Diego.
- Plock, N., and Kloft, C. (2005) Microdialysis--theoretical background and recent implementation in applied life-sciences, *Eur J Pharm Sci* 25, 1-24.
- Titier, K., Castaing, N., Scotto-Gomez, E., Pehourcq, F., Moore, N., and Molimard, M. (2003) Titier, K. et al. High-performance liquid chromatographic method with diode array detection for identification and quantification of the eight new antidepressants and five of their active metabolites in plasma after overdose *Ther Drug Monit* 25, 581-587.
- Trabace, L., Cassano, T., Cagiano, R., Tattoli, M., Pietra, C., Steardo, L., Kendrick, K., and Cuomo, V. (2001) Trabace, L. et al. Effects of ENA713 and CHF2819, two anti-Alzheimer's disease drugs, on rat amino acid levels, *Brain Res.* 910, 182-186.
- Ungerstedt, U. (1991) Microdialysis--principles and applications for studies in animals and man, *J Intern Med* 230, 365-373.
- Yoshitake, T., Yamaguchi, M., Nohta, H., Ichinose, F., Yoshida, H., Yoshitake, S., Fuxe, K., and Kehr, J. (2003) Yoshitake, T. et al. Determination of histamine in microdialysis samples from rat brain by microbore column liquid chromatography following intramolecular excimer-forming derivatization with pyrene-labeling reagent *J Neurosci Methods* 127, 11-17.
- Zhang, M. Y. B., C.E. (2006) Measurement of neurotransmitters from extracellular fluid in brain by in vivo microdialysis and chromatography-mass spectrometry *J Pharm Biomed Anal* 40, 492-499.

Zhu, Y., Wong, P., Cregor, M., Gitzen, J., Coury, L., and Kissinger, P. (2000) Zhu, Y. et al. In vivo microdialysis and reverse phase ion pair liquid chromatography/tandem mass spectrometry for the determination and identification of acetylcholine and related compounds in rat brain. *Rapid Commun Mass Spectrom Rapid Commun Mass Spectrom* 14, 1695-1700.

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