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Pharmacokinetic and pharmacodynamic analyses of trazodone in rat striatum by in vivo microdialysis

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Abstract

The aim of this study was to investigate the brain pharmacokinetics and pharmacodynamics of trazodone. Sensitive microbore high-performance liquid chromatographic methods with electrochemical detection (LC-ED) were developed for the determination of trazodone, serotonin (5-HT), and their respective metabolites. The feasibility of microdialysis coupled with LC-ED system for direct analysis of these compounds in the rat striatum was investigated. Striatal dialysates were automatically injected onto a cyano microbore column, through an on-line injector, for the determination of trazodone and its metabolite or onto a reversed phase microbore column for the determination of 5-HT and its metabolite. A monophase phenomenon with a first-order elimination rate constant was observed for trazodone. The brain pharmacokinetics of trazodone appear to conform to a one-compartment model. Surprisingly, no significant changes in striatal 5-HT or its metabolite were observed following the same dosage and time course. The present results suggest that brain microdialysis methods may be applicable to pharmacokinetic and pharmacodynamic studies of psychotrophic agents.

Keywords: Pharmacodynamic; Brain; Trazodone

1. Introduction

Trazodone, 2,3-(4-*m*-chlorophenyl-1-piperazinyl)propyl-1,2,4-triazol-4,3-pyridin-3(2H)-one, is a triazolopyridine derivative compound which is a specific inhibitor of the synaptosomal uptake of serotonin (5-HT) [1,2]. Although trazodone increases 5-HT in the brain, its metabolite, 1-*m*-chlorophenylpiperazine (*m*-CPP), acts as a 5-HT_{1A} agonist decreasing 5-HT release. Therefore, the measurements of trazodone, 5-HT and their metabolites in the brain are important for studying the psychotrophic role of trazodone.

Several methods have been reported for the determination of trazodone such as LC equipped

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with ultraviolet detection [3,4], fluorescence detection [5,6], or electrochemical detection (ED) [7,8]. Among these, LC-ED is considered to be one of the most sensitive for the determinations of trazodone and *m*-CPP. However, no simple and satisfactory LC method exists for the simultaneous determination of trazodone and *m*-CPP in small volumes of sample. Current methods require extensive and time-consuming cleanup procedures [3–5,8].

A sensitive LC-ED method is also required to detect 5-HT in rat striatal dialysates which is present at low-picogram or even sub-picogram levels. The low basal 5-HT concentrations in rat striatal dialysates were successfully measured via a method, which employed a microbore LC-ED system developed in this laboratory [9]. Thus, striatal 5-HT and its metabolite, 5-HIAA and their pharmacodynamic responses to trazodone can be evaluated.

Recent advances in brain microdialysis techniques have enabled the direct measurement of various neurotransmitters in the brain [10,11]. However, pharmacokinetic investigations of psychotropic drugs are limited using this method [12–15]. Since drug monitoring is crucial for the rational therapeutic use of drugs, the feasibility of employing the brain microdialysis method for pharmacokinetic studies is of particular importance [12]. In addition, the effects of pharmacological dosages of trazodone and *m*-CPP on the release of 5-HT on the brain are unclear. Therefore, the simultaneous measurement of 5-HT and its metabolite, 5-HIAA, in the brain is an important research strategy for studying the pharmacodynamic role of psychotropic or serotonergic drugs.

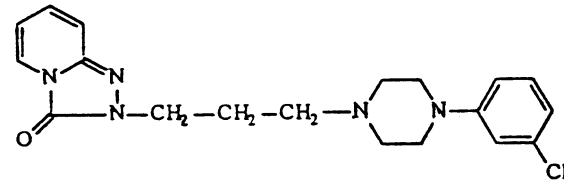
2. Experimental section

2.1. Drugs and chemicals

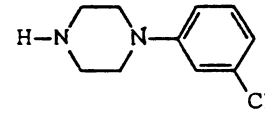
Trazodone and 1-*m*-chlorophenylpiperazine (*m*-CPP) (Fig. 1) were purchased from RBI (Natick, MA). 5-hydroxyindoleacetic acid (5-HIAA) and 5-HT were purchased from Sigma (St Louis, MO), prepared at a concentration of 2 ng ml^{-1} in 0.1 M perchloric acid, stored at −70°C in the dark and thawed in an ice bath prior to preparation of a standard mixture. Methanol, acetonitrile, and orthophosphoric acid were obtained from E. Merck (Merck–Schuchardt, Darmstadt, Germany). Triple deionized water (Millipore, Bedford, MA) was used for all preparations.

2.2. Microdialysis

Adult male Sprague–Dawley rats (300–350 g) were initially anesthetized with Na pentobarbital (50 mg kg^{-1} i.p.) [16]. Each rat was cannulated with PE-50 at the right femoral vein for drug administration. After femoral vein cannulation, each rat was placed in a Kopt sterotaxic frame and its body temperature was maintained at 37°C with a heating pad. A microdialysis probe (CMA-12; CMA/Microdialysis AB) with a tip length of 4 mm and an outer diameter of 0.5 mm was implanted into the right striatum with the tip located at AP 0.4 mm, ML −3.0 mm, DV −7.0 mm, from bregma and dura surface, respectively [16]. The probes were perfused with Ringer's solution (147 mM Na^+, 4.0 mM K^+, 2.2 mM Ca^{2+}) at a flow-rate of 1 μl min^{-1}, by a microinjection pump (CMA-100). The dialysis sample flowed to an on-line injector (CMA-160) and LC-ED or was collected in a microfraction collector (CMA-140).



A



B

Fig. 1. Chemical structures of A: trazodone and B: *m*-CPP.

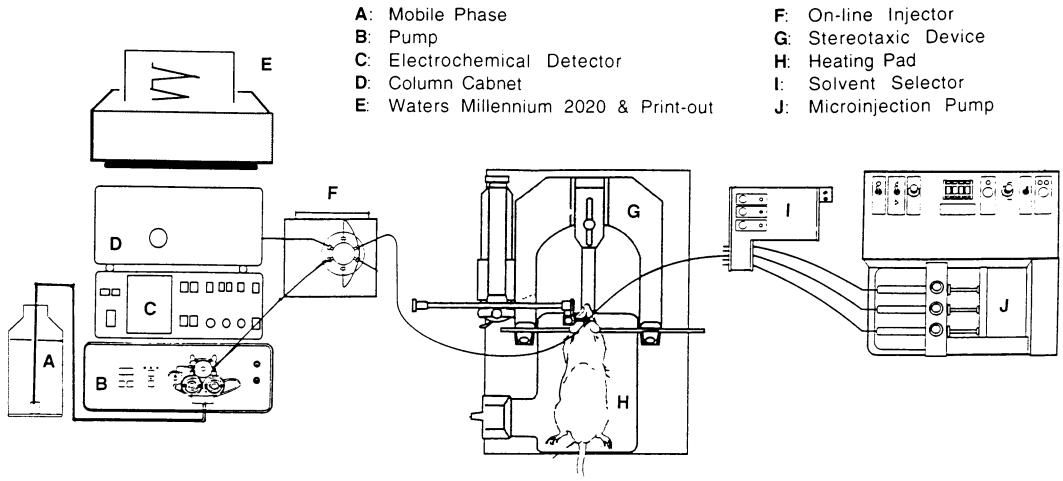


Fig. 2. An on-line microdialysis system includes: A: Mobile phase; B: Pump; C: Electrochemical Detector; D: Column Cabnet; E: Computer Print-out; F: On-line Injector; G: Stereotaxic Device; H: Heating Pad I: Solvent Selector; and J: Microinjection Pump.

2.3. Chromatographic system

The LC-ED system was comprised of a chromatographic pump (BAS, PM-80, Bioanalytical System, West Lafayette, IN, USA) set at flow-rate 70 μl min^{-1} for trazodone and *m*-CPP analyses, acyano microbore column (BAS SepStik-CN, 150 × 1.0 mm i.d., particle size 5 μm) and an on-line injector. The mobile phase consisted of 0.1 M monosodium dihydrogen orthophosphate–methanol–diethylamine (80:20:0.1, v/v/v, pH 3.0 adjusted with orthophosphoric acid). The mixture was filtered with a 0.22 μm Millipore membrane and degassed by helium. The injection volume was configured with a 10 μl sample loop on an on-line injector as shown in Fig. 2 (CMA-160, CMA/Microdialysis AB, Stockholm, Sweden). Trazodone and *m*-CPP were detected using an amperometric detector (BAS, LC-4C) coupled to a glassy carbon working electrode and referenced to a Ag/AgCl electrode at + 1.10 V. Output from ED was amplified and recorded using Waters Millennium 2020 software.

Dialysates (5 μl) were directly injected onto a microbore LC-ED system with an amperometric detector (BAS-4C and the MF-1020 electrode, Bioanalytical, West Lafeyette, IN) to measure 5-HT and 5-HIAA. The potential was set at + 0.75 V with respect to a silver/silver chloride reference electrode [9]. Separation of these substances was achieved using a microbore column (150 × 1.0 mm i.d.) packed with 5 μm Inertsil-2 C_{18} particles (GL, Tokyo, Japan). The mobile phase consisted of 50 ml acetonitrile and 950 ml of monochloroacetic acid solution (9.60 g monochloroacetic acid, 0.16 g sodium 1-octane sulfonate, 10 mg ethylenediaminetetraacetic acid in 1 l triple deionized water and adjusted to pH 3.0 with 1 M sodium hydroxide) [9]. The flow-rate was 60 μl min^{-1}. The concentrations of 5-HT and 5-HIAA concentrations in dialysates were calculated by determining each peak area ratio relative to the standard mixture.

2.4. Pharmacokinetic study

Calibration curves were constructed based on LC analyses of various concentrations of trazodone and *m*-CPP (5–100 ng ml^{-1}). The concentrations of trazodone and *m*-CPP in rat brain dialysates were determined from the calibration curves. Following a 2-h period of stabilization, trazodone was administered (5 mg kg^{-1}, i.v.). The catheter was then immediately flushed with 0.5 ml normal saline. During the preparation, the first interval of 0–10 min was discarded to eliminate the void volume of the on-line system. Dialysates were collected and injected every 10 min

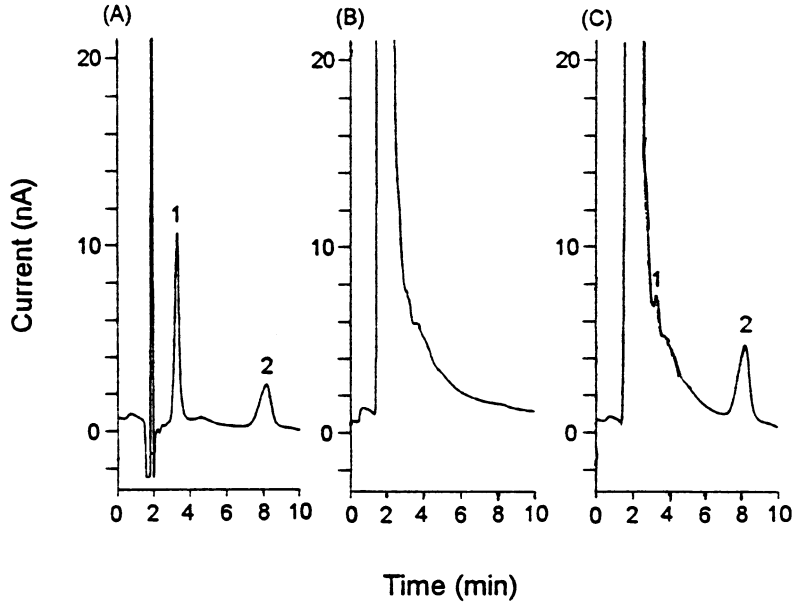


Fig. 3. Typical chromatograms of (A) authentic trazodone and *m*-CPP (50 ng ml^{-1} each), (B) blank striatal dialysate, and (C) trazodone (79.51 ng ml^{-1}) and *m*-CPP (1.97 ng ml^{-1}) in dialysate samples from rat striatum after 30 min of trazodone (5 mg kg^{-1}, i.v.) administration. (1: *m*-CPP; 2: trazodone.)

for an additional 3 h after trazodone administration. All concentration–time data were processed by the computer program 'PCNONLIN' (SCI, Lexington, KY), with reciprocal concentration weights (1/C) for pharmacokinetic parameter calculations.

2.5. Pharmacodynamic study

Following a 2-h period of stabilization and for 3 h after trazodone administration (5 mg kg^{-1}, i.v.), dialysates from another group of rats ($n = 6$) were collected in a CMA-140 microfraction collector and injected onto a microbore LC-ED. The concentrations of 5-HT and 5-HIAA in rat brain dialysates were determined as previously described [9].

3. Results

On typical chromatograms, the retention times of *m*-CPP and trazodone were 3.5 and 8.2 min, respectively (Fig. 3). Fig. 3(A) shows standard samples of trazodone (50 ng ml^{-1}) and its metabolite, *m*-CPP (50 ng ml^{-1}). Fig. 3(B) shows the chromatogram of a blank brain dialysate. No discernible peaks were observed within the time frame in which trazodone and *m*-CPP were detected. Fig. 3(C) shows a chromatogram of a dialysate sample containing *m*-CPP (1.97 ng ml^{-1}) and trazodone (79.51 ng ml^{-1}), obtained from brain microdialysis 30 min after trazodone administration. The concentrations of *m*-CPP in dialysate which varied in the range of approximately 1–2 ng ml^{-1}. Unfortunately, the detection limit of *m*-CPP was about 1 ng ml^{-1}. Therefore, these *m*-CPP data were around the detection limit or slightly above it. These data can only be accepted with reservation.

In Table 1, the in vitro recoveries of trazodone (50 ng ml^{-1}), *m*-CPP (50 ng ml^{-1}), 5-HT (10 ng ml^{-1}), and 5-HIAA (10 ng ml^{-1}) from the microdialysis probes ($n = 6$), were 25, 24, 24, and 23%, respectively. The reproducibilities of the methods were also defined by examining both intra- and inter-assay variabilities. The intra-assay ($n = 8$) variabilities for the determination of trazodone

Table 1
Analytical precision (C.V. values, %) of various standard mixtures on the intra-assays and inter-assays of the measuements of 5-HT, 5-HIAA, trazodone, and *m*-CPP

ng ml^{-1}	5-HT	5-HIAA	ng ml^{-1}	Trazodone	*m*-CPP
Intra-assay ($n=25$)			Intra-assay ($n=8$)		
0.2	5.8	6.5	10	8.3	8.8
2.0	4.2	2.9	20	5.3	5.1
10	3.8	1.6	50	5.7	5.0
Inter-assay ($n=6$)			Inter-assay ($n=6$)		
0.2	4.5	6.8	10	9.5	8.9
2.0	4.2	5.5	20	6.2	6.3
10	3.8	4.9	50	4.0	4.3
In vitro recovery ($n=6$)					
10	24%	23%	50	25%	24%
Detection limit S/N = 3	0.01	0.01		1	1

C.V. = (standard deviation/mean concentration) × 100%

and *m*-CPP at concentrations of 10, 20, and 50 ng ml^{-1} were acceptable with coefficients of variation values (CVs) of less than 9%. The inter-assay CVs ($n=6$) for trazodone and *m*-CPP at the same concentrations were less than 10%. The detection limit for trazodone and *m*-CPP, at a signal-to-noise ratio of 3, was about 1 ng ml^{-1}. In the standard mixtures of 5-HT and 5-HIAA (0.2–10 ng ml^{-1}), the CVs ($n=25$) were acceptable ($<$ 7%). The inter-assay CVs ($n=6$) for 5-HT and 5-HIAA at the same concentrations were less than 5%. The detection limit was 0.01 ng ml^{-1} for 5-HT and 5-HIAA.

Fig. 4(A) shows a typical chromatogram of the authentic standard samples of 5-HT (20 ng ml^{-1}) and its metabolite, 5-HIAA (20 ng ml^{-1}). Fig. 4(B) shows a typical chromatogram of a dialysate sample containing 5-HT (0.29 ng ml^{-1}) and 5-HIAA (42.14 ng ml^{-1}) obtained from brain microdialysis 30 min after trazodone administration. The concentrations of 5-HT and 5-HIAA in dialysates sampled from the striatum ($n=6$) of rats subjected to trazodone (5 mg kg^{-1}, i.v.) administration are shown in Fig. 5. The samples were collected at 10 min intervals during the entire experimental period.

The concentrations of trazodone and *m*-CPP in dialysates, sampled from the striatum ($n=6$) of rats subjected to trazodone (5 mg kg^{-1}, i.v.) administration, are shown in Fig. 6. The samples were collected at 10 min intervals during the entire experimental period. The data were compared with pharmacokinetic models (one- versus two-compartment) according to Akaike's information criterion (AIC) [17] and Schwarz criterion (SC) [18], with minimum AIC and SC values being regarded as the best representation of the plasma concentration–time course data. A one-compartment open model with individual animal data after each dose was proposed by the computer program PCNONLIN. The following equation applies to a one-compartment pharmacokinetic model:

$$C = A\ \mathrm{e}^{-at} \quad (1)$$

In Eq. (1), A is the concentration (C) intercept for fast or slow disposition phase, and a is the disposition rate constant for the disposition phases. Analysis of data after i.v. injection of trazodone (5 mg kg^{-1}) yielded Eq. (2).

$$C = 136\ \mathrm{e}^{-0.022t} \quad (2)$$

The brain pharmacokinetic parameters, as derived from the data and calculated by PCNONLIN program, are shown in Table 2.

4. Discussion

In a previous paper, Suckow [7] described the simultaneous determination of trazodone and *m*-CPP in plasma, using two internal standards coupled with a dual recorder set at different attenuations. However, with this method there may be a difficulty in the separation of trazodone, *m*-CPP and internal standards when duplicated in other laboratories. Ohkubo et al. [8] described an improved LC method coupled with two detectors, an ultraviolet detector and an electrochemical detector, for the determination of diverse concentrations of trazodone and *m*-CPP. Both methods require either complicated instrumentation or tedious sample preparation procedures. The present method was aimed at the simultaneous measurement of trazodone and *m*-CPP using on-line microdialysis with a microbore LC-ED. An efficient clean-up by microdialysis, with a cyano microbore column, eliminates large endogenous molecules and provides clean chromatograms and well-resolved trazodone and *m*-CPP peaks (Fig. 3).

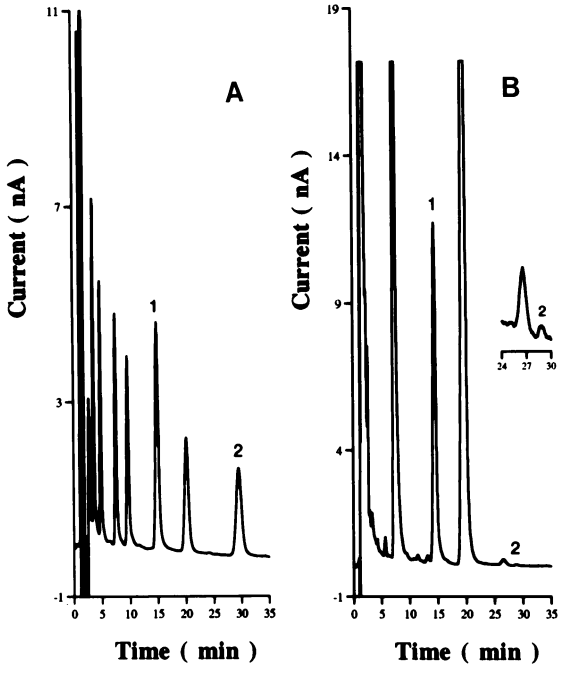


Fig. 4. Typical chromatograms of (A) authentic 5-HIAA and 5-HT(20 ng ml^{-1} each) (B) 5-HIAA (42.14 ng ml^{-1}) and 5-HT (0.29 ng ml^{-1}) in dialysate samples from rat striatum after 30 min of trazodone (5 mg kg^{-1}, i.v.) administration. (1: 5-HIAA; 2: 5-HT.)

Compared to conventional LC systems [8], the cyano microbore column results in small band broadening of analytes, so that sharper peaks are obtained under optimal chromatographic conditions. The lower flow-rates in a microbore LC-ED system provide a smoother baseline to achieve low detection limits. Also, in a microbore LC-ED system, the time analytes are in contact with the working electrode is prolonged, resulting in high coulometric yields [19,20]. Hence, the microbore LC system enhances detection sensitivity and achieves optimum detection limits. Furthermore, microbore LC-ED systems require only small quantities of samples, similar to the quantities required by microdialysis sampling methods. In addition, on-line analysis (Fig. 2) improves analytical reproducibility and avoids the need for preservatives in the samples and tedious manual procedures.

Previous papers have illustrated that plasma concentrations of trazodone and *m*-CPP are at diverse levels [4,7,8]. In general, *m*-CPP levels are less than 10% of trazodone levels in plasma. However, Miller et al. [4] reported that *m*-CPP was a predominant metabolite in brain tissue. Their measured *m*-CPP levels were about the same or even higher than trazodone levels in brain tissue. These data might be interpreted to mean that there are different metabolic rates or functions in the central and peripheral compartments. The optimal therapeutic range for trazodone and the adverse contribution of *m*-CPP, therefore, cannot be established from blood pharmacokinetic data alone, and caution is needed in interpreting pharmacodynamic effects of trazodone in the central nervous system. In our study, trace *m*-CPP levels (ca. $< 5\%$ of trazodone) were found in brain microdialysates. The discrepancies between our data and data from Miller's group [4] may be due to differences in sampling assays. Their data, obtained from brain homogenates, represent total trazodone and *m*-CPP, whereas our data represent unbound trazodone and *m*-CPP. Nevertheless, our data did not support the conclusion that *m*-CPP is a predominant metabolite of trazodone

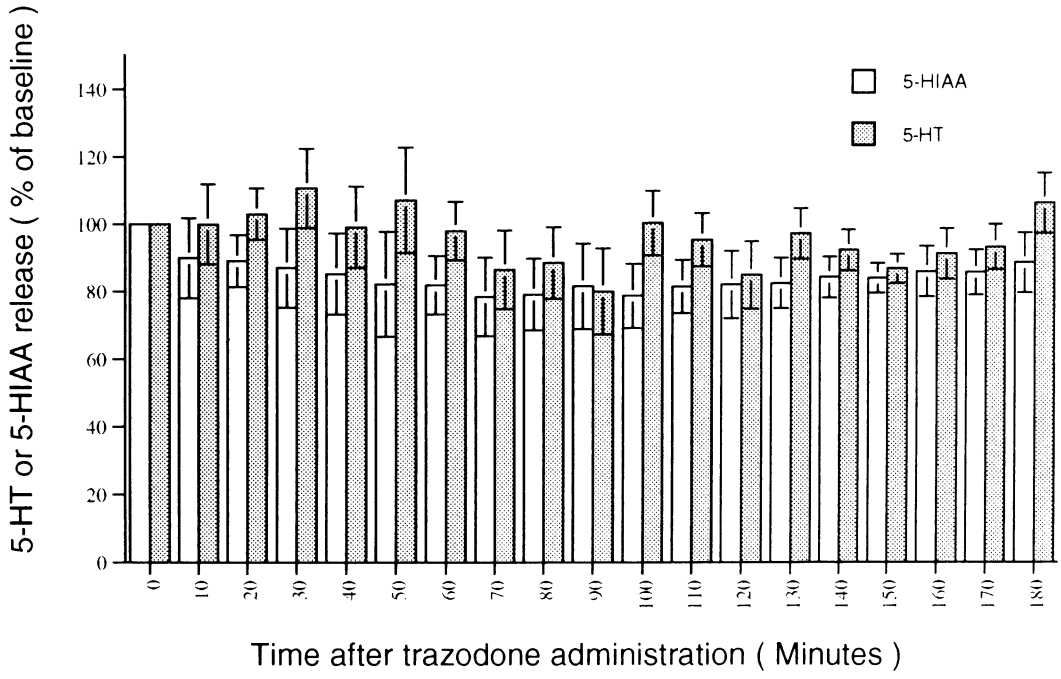


Fig. 5. Time course of changes in dialysate 5-HT and 5-HIAA in rat striatum ($n = 6$) subjected to trazodone (5 mg kg^{-1}, i.v.) administration. The samples were collected at 10 min intervals during the entire experimental period.

in brain. Further investigations are needed to explore the metabolism of trazodone and *m*-CPP in brain.

It was assumed on the basis of in vitro studies that trazodone or *m*-CPP activate the serotonergic system. It has been shown that *m*-CPP inhibits release of endogenous 5-HT from synaptosomal and brain slice preparation. In this study, there were no significant 5-HT or 5-HIAA levels changes following administration of trazodone for at least 3 h. This is the first study on the effects of trazodone on striatal 5-HT and 5-HIAA. There was no evidence to support a specific serotonin release which responds preferentially to trazodone.

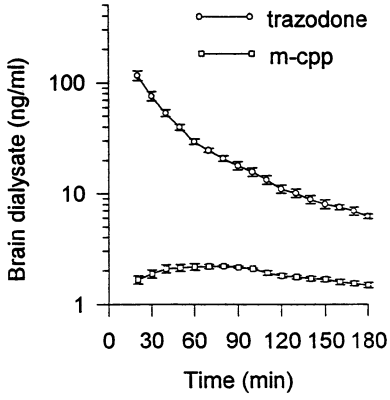


Fig. 6. The concentrations of trazodone and *m*-CPP in dialysates, sampled from the striatum ($n = 6$) of rats subjected to trazodone (5 mg kg^{-1}, i.v.) administration. The samples were collected at 10 min intervals during the entire experimental period.

Table 2
Pharmacokinetic parameters of trazodone (5 mg kg^{-1}, i.v.) in rat brain striatum

Parameters	Estimate
A, ng ml^{-1}	136 ± 18.2
a, l min^{-1}	0.022 ± 0.002
AUC, ng min ml^{-1}	6130 ± 380
1/2, min	33.2 ± 3.7
AUMC, ng min^2 ml^{-1}	288 000 ± 22 000
MRT, min	47.8 ± 5.4

Data are expressed as mean ± S.E.M. ($n = 6$).

5. Conclusion

The present microbore LC-ED was applied to simultaneously determine trazodone, 5-HT, and their metabolites in rat striatal dialysates. The on-line microdialysis system was used to investigate rats receiving an i.v. administration of trazodone. The detection limit for trazodone and *m*-CPP, at a signal-to-noise ratio of 3, was about 1 ng ml^{-1} (about 0.01 ng ml^{-1} for 5-HT and 5-HIAA). A monophase phenomenon with a first-order elimination rate constant for trazodone was observed from the brain dialysate concentration–time curve. The results indicate that the brain pharmacokinetics of trazodone conform to a one-compartment model. Unlike in brain tissue, *m*-CPP is a trace metabolite of trazodone in the brain striatum. No changes in 5-HT or 5-HIAA were observed. It seems unlikely that the serotonergic system is activated at a dosage of 5 mg kg^{-1} trazodone via i.v. administration. However, further investigations are needed to clarify the metabolism of trazodone in brain. In addition, the effects of varied dosages of trazodone on brain serotonergic system need to be investigated. The present results suggest that the brain microdialysis method may be applicable to further pharmacokinetic and pharmacodynamic studies of psychotropic or neurotrophic agents.

Acknowledgements

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