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Note

Separation and determination of sertraline and its metabolite, desmethylsertraline, in mouse cerebral cortex by reversed-phase high-performance liquid chromatography

HARVEY L. WIENER*

Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, St. John's University, Jamaica, NY 11439 (U.S.A.)

and

H. KENNETH KRAMER and MAARTEN E.A. REITH

Center for Neurochemistry, The Nathan S. Kline Institute for Psychiatric Research, Ward's Island, NY 10035 (U.S.A.)

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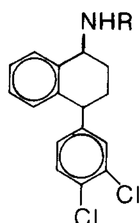
Sertraline [(1*S*,4*S*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl-1-naphthylamine; Fig. 1] is a member of the latest generation of antidepressant



Sertraline R=CH_3
Desmethylsertraline R=H

Fig. 1. Structures of sertraline and desmethylsertraline.

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agents that selectively inhibit serotonin uptake in brain [1,2]. Chronic administration of sertraline causes subsensitivity of β-adrenergic receptors in the brain [3], an effect common to many antidepressant drugs of varying pharmacological classes [4]. Consonant with the involvement of serotonin in sertraline's effect is the observed potentiation of L-5-hydroxytryptophan-induced head twitches in mice [5]. Sertraline's lack of anticholinergic and cardiotoxic effects makes it a potentially beneficial antidepressant for elderly patients [6], and a study in non-depressed volunteers of 50 years and over showed sertraline to be superior to amitriptyline in a psychomotor performance test [7]. In addition to being an effective antidepressant without anticholinergic, antihistaminic, and sympathomimetic side-effects, sertraline may be beneficial for the treatment of affective disorders and obsessive compulsive behavior [2].

Sertraline is metabolized via N-demethylation to desmethylsertraline (Fig. 1), the primary metabolite [8]. Pharmacokinetic and behavioral studies in our laboratory necessitated the development of a method for the separation and determination of sertraline and desmethylsertraline in mouse cerebral cortex. Although a gas chromatographic–mass spectrometric (GC–MS) method was described by Fouda et al. [8] for the determination of sertraline in human plasma, their method requires extensive sample preparation and fails to elute desmethylsertraline from the column employed. Saletu et al. [9] determined the plasma concentration of sertraline and desmethylsertraline in human volunteers receiving three different oral doses of sertraline. Their study employed a GC method with an electron-capture detector; however, since they did not provide the reader with the necessary details as to sample preparation, extraction, derivatization, and chromatographic conditions it is difficult to determine the general applicability of their method.

The present communication describes a rapid, isocratic reversed-phase high-performance liquid chromatographic (HPLC) method that requires minimal sample preparation and is the first HPLC method to quantify both sertraline and desmethylsertraline simultaneously. The present study also describes preliminary pharmacokinetic data for sertraline and desmethylsertraline in mouse cerebral cortex following a single intraperitoneal (i.p.) injection of sertraline.

EXPERIMENTAL

Materials

The following HPLC-grade chemicals were purchased from Fisher Scientific (Fairlawn, NJ, U.S.A.): monobasic potassium phosphate, phosphoric acid, acetonitrile, and ethanol. Sertraline hydrochloride was provided by Pfizer Pharmaceuticals (New York, NY, U.S.A.). Desmethylsertraline maleate was generously provided by Dr. L.M. Tremaine (Pfizer Central Research, Groton,

CT, U.S.A.). Tetracaine hydrochloride was purchased from Sigma (St. Louis, MO, U.S.A.).

Animals and administration of sertraline

Male C57BL/6ByJ mice, 10–12 weeks old, with a mean weight of 24 ± 0.3 g, from the Center for Neurochemistry breeding colony were used. The mice were kept on a 12 h light–12 h dark cycle, with food and water available ad libitum. Sertraline hydrochloride was dissolved in distilled water and injected i.p. in a volume of 0.2 ml per 20 g body weight.

Extraction and reversed-phase HPLC

The mice were killed by decapitation, the brain was rapidly removed, and the cerebral cortex dissected and weighed (average wet weight was approximately 130 mg). The cerebral cortex was then placed in a polypropylene tube and 1.1 ml of ethanol was added. Tetracaine hydrochloride was added as an internal standard to a final concentration of 10 *μM*. The cerebral cortex was then disrupted by sonication. Following centrifugation, 20 μl of the supernatant were injected onto a Versapack C_{18} column (10 μm, 300 mm × 4.1 mm I.D.; Alltech Assoc., Deerfield, IL, U.S.A.). Elution was performed with equipment purchased from Bio-Rad Labs. (Richmond, CA, U.S.A.; pump Model 133) at room temperature. An isocratic mobile phase consisting of 0.25 *M* potassium phosphate buffer (pH 2.7) containing 30% (v/v) acetonitrile was used. The flow-rate was 2 ml/min and the absorbancy at 235 nm was monitored using a Bio-Rad UV detector (Model 1305A).

RESULTS AND DISCUSSION

Fig. 2A shows a typical chromatogram for an aqueous standard solution of tetracaine (peak 1), desmethylsertraline (peak 2), and sertraline (peak 3). Tetracaine, the internal standard, eluted at 4.9 min. Desmethylsertraline and sertraline were completely resolved and eluted at 9.5 and 12 min, respectively. Standard curves for sertraline and desmethylsertraline were linear and had the following regression coefficients: for sertraline: slope = 0.105, *y*-axis intercept = −0.8, $r^2 = 0.998$; for desmethylsertraline: slope = 0.11, *y*-axis intercept = 1.37, $r^2 = 0.989$ (Fig. 3).

When the cerebral cortex from control mice that received vehicle alone was extracted as described in the Experimental section, the chromatogram was free of interference in the regions where desmethylsertraline and sertraline eluted (Fig. 2B).

Fig. 2C shows a chromatogram of a sample from a control mouse injected with vehicle alone and spiked with tetracaine, desmethylsertraline, and sertraline. A recovery study for sertraline and desmethylsertraline additions to mouse cerebral cortex showed that after one extraction with ethanol, 89% of the ser-

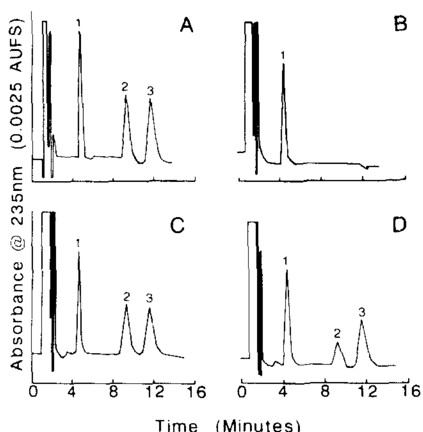

Fig. 2. Typical reversed-phase HPLC elution profiles for sertraline and desmethylsertraline. (A) Aqueous standard solution of tetracaine (200 pmol injected; peak 1), desmethylsertraline (250 pmol injected; peak 2), and sertraline (250 pmol injected; peak 3). (B) Control animal that received vehicle only. (C) Sample from a control animal spiked with tetracaine, sertraline, and desmethylsertraline. (D) Animal killed 30 min after receiving 32 mg/kg sertraline hydrochloride.

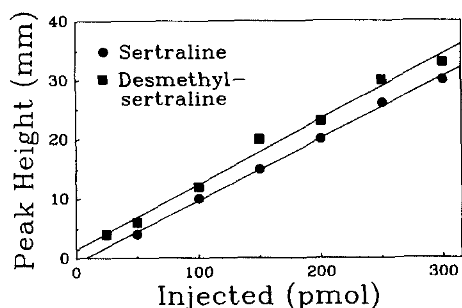

Fig. 3. Representative standard curves for sertraline and desmethylsertraline.

TABLE I

RECOVERY AND WITHIN-DAY PRECISION

Compound	Added[a] (nmol)	Determined (mean ± S.E.M., $n=10$) (nmol)	Recovery (%)	Coefficient of variation[b] (%)
Sertraline	14.75	13.2 ± 0.2	89	4.8
Desmethylsertraline	14.75	12.8 ± 0.2	87	4.9

[a]Sertraline or desmethylsertraline was added to cerebral cortex samples (130 mg average wet weight) and extracted as described in the Experimental section.
[b]Coefficient of variation (%) = (standard deviation/mean) × 100.

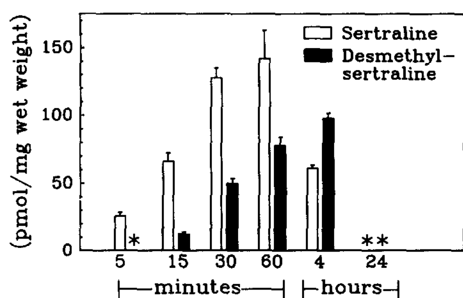

Fig. 4. Time course for sertraline and desmethylsertraline in the cerebral cortex of male C57BL/6ByJ mice which received sertraline hydrochloride (32 mg/kg i.p.). The results are expressed as the mean ± S.E.M (six animals per time point). Desmethylsertraline and/or sertraline were not detected where indicated by the asterisk.

traline and 87% of the desmethylsertraline added was recovered (Table I). The coefficient of variation for sertraline and desmethylsertraline was 4.8 and 4.9%, respectively.

After i.p. administration of 32 mg/kg sertraline, there was a rapid appearance of sertraline in the cerebral cortex, detectable as early as 5 min after injection (Fig. 4). This dosage was chosen for two reasons: first, behavioral studies in our laboratory have shown that this is the necessary dose to shift the time-course of cocaine-induced locomotion in C57BL/6ByJ mice [10], and secondly, to determine if any sertraline or desmethylsertraline remains 24 h after this dose an important issue in long-term experiments with daily sertraline injections. The level of sertraline increased up to 1 h, was on the decline at 4 h, and was below the detection limit 24 h after a single injection. The limit of detection for sertraline under the conditions employed was approximately 20 pmol/mg wet weight. To determine the limit of detection, serial dilutions of sertraline (or desmethylsertraline) were added to cerebral cortex samples from vehicle-treated animals and the samples extracted as described above; the limit of detection was estimated by the minimal concentration of sertraline (or desmethylsertraline) which could be distinguished from a suitable blank (Fig. 2B). The sensitivity of this procedure can be easily increased by either extracting more tissue, extracting in a smaller volume of ethanol, or injecting a larger volume of the extract onto the reversed-phase HPLC column employed. These modifications would also allow quantification of sertraline and desmethylsertraline after lower doses of sertraline. Although the present communication describes an extraction and chromatographic procedure for mouse cerebral cortex, this method can be readily modified for quantification of sertraline and desmethylsertraline in smaller brain regions or in the rat.

Desmethylsertraline was first observed 15 min after administration of sertraline. The level of desmethylsertraline was highest at the 4-h time point and was below the detection limit after 24 h. The limit of detection of desmethyl-

sertraline was approximately 10 pmol/mg wet weight. Fig. 2D shows a typical chromatogram at the 30-min time point. The observation that the concentration of desmethylsertraline in the brain was still increasing in a time period (1–4 h) when the sertraline level was already declining indicates that the uptake of desmethylsertraline into (or its clearance from) the brain is slower than that of sertraline. Although desmethylsertraline is eight times less potent than sertraline in inhibiting serotonin uptake [6], the relatively high levels of desmethylsertraline at longer times may contribute to the blockade of serotonin uptake and explain why the decline in this blockade from 1 to 4 h after i.p. sertraline administration is less pronounced [1] than the decline in brain levels of sertraline (Fig. 4). The plasma contribution of sertraline (or desmethylsertraline) to the concentration of sertraline (or desmethylsertraline) in the cerebral cortex was negligible at all time points because determination of plasma samples in animals at the 60-min time point could not detect sertraline (or desmethylsertraline); with a plasma limit of detection of 25 and 12 μM for sertraline and desmethylsertraline, respectively, it can be calculated that under the conditions used the maximal contribution of plasma sertraline or its metabolite to estimated brain levels is negligible.

In summary, this paper reports a rapid and sensitive isocratic reversed-phase HPLC method for the determination of sertraline and desmethylsertraline in brain tissue. The procedure involves minimal sample preparation and allows simultaneous quantification of the two compounds.

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