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Note

High-performance liquid chromatographic determination of tetrahydroaminoacridine in human and rat tissues using a rapid Sep-Pak C_{18} extraction

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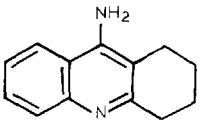
Tetrahydroaminoacrin (THA, 9-amino-1,2,3,4-tetrahydroacridine), I, is a competitive cholinesterase inhibitor [1]. It has been used clinically for the treatment of intractable pain of terminal carcinoma [2], myasthenia gravis [3], and as a decurarizing agent [4]. The drug has also been utilized as a specific therapy for reversing the signs and symptoms of the central anticholinergic syndrome [5]. Particular interest in this compound derives from its possible efficacy in patients with Alzheimer's disease [6, 7].



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THA has been administered in variable doses ranging from 0.25 to 1.5 mg/kg. It has a duration of action from 8 to 16 h [8]. There are considerable inter-individual variations in adverse reactions. Hence, an assay of the plasma concentration of THA would be an useful adjunct to its use in elderly patients with Alzheimer's disease.

Yago et al. [9] used high-performance liquid chromatography (HPLC), utilizing a two-step extraction procedure for sample purification, to determine

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THA levels in spiked plasma samples. More recently, small packed (Sep-Pak) cartridges have been used [10, 11] for the purification of biological samples prior to HPLC.

In the present paper, we have employed our previously developed purification technique [10] for the isolation of THA from plasma and brain samples. THA was separated and quantified by reversed-phase HPLC with UV absorbance detection. This method has been successfully utilized in pharmacokinetic studies of rats administered THA intraperitoneally and is applicable for the determination of THA in human plasma.

EXPERIMENTAL

Apparatus

An LC-306 liquid chromatograph (Bioanalytical Systems, West Lafayette, IN, U.S.A.) was used throughout this work. A 30 cm × 4 mm μBondapak C_{18} reversed-phase column (Waters Assoc., Milford, MA, U.S.A.) was connected to a 3 cm × 4.6 mm guard column of 5-μm RP-18 (Brownlee Labs., Santa Clara, CA, U.S.A.). A Waters Model M420 fluorometric detector (excitation 385 nm/emission 425 nm) was used to confirm the identity of the THA peak observed in plasma and brain samples. The mobile phase was prepared by mixing 1600 ml of 0.1 *M* phosphoric acid solution (adjusted to pH = 2.8 by triethylamine) and 200 ml of acetonitrile. The flow-rate was fixed at 1.5 ml/min at ambient temperature and the UV wavelength set at 254 nm.

Reagents

THA standard was obtained from Sigma (St. Louis, MO, U.S.A.). C_{18} Sep-Pak cartridges were supplied by Waters Assoc. Acetonitrile and methanol were purchased from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). All chemicals were reagent grade.

Sample preparation

Male, Sprague—Dawley rats (Charles River, MA, U.S.A.) weighing 200—300 g were used for the studies. THA was dissolved in saline and administered intraperitoneally in a dose of 20 mg/kg. Control animals were injected with saline.Blood was drawn at 5, 25, 30, 45 and 60 min following the THA injection by an open chest cardiac puncture. The blood was centrifuged at 2000 *g* (4°C) for 20 min and plasma carefully removed. Immediately upon completion of the cardiac puncture, each rat was decapitated by guillotine and the whole brain rapidly removed. Brain tissue samples were weighed and homogenized in propylene tubes with 0.2 *N* perchloric acid (1 : 2, g/ml). The homogenates were then centrifuged at 12,000 *g* for 20 min at 4°C. The clear supernatant was adjusted to a pH of 7.2 by adding 1 *N* sodium hydroxide. Human plasma samples were obtained from the hospital blood bank.

THA isolation procedure

The C_{18} Sep-Pak cartridge was activated by passing through 5 ml of water and then 5 ml of methanol under pressure using a glass syringe followed by 10 ml of water. A 1-ml aliquot of rat plasma or brain homogenate was passed

through the cartridge via a syringe at a flow-rate not greater than 2 ml/min. The cartridge was washed with 10 ml of water and 4 ml of the mobile phase. The residue solution was evacuated by pushing a plunger through the syringe. Methanol (0.3 ml) was added to the cartridge and the evacuation procedure repeated. An additional 0.7 ml of methanol was passed through the cartridge and the eluate collected in a 10-ml glass disposable tube. The methanol was evaporated to dryness under a stream of dry nitrogen at 35°C. The dried residue was dissolved in 100 μl of the mobile phase. Ten μl were injected into the chromatograph. The cartridge can be regenerated by flushing with 10 ml of methanol and 15 ml of water. The concentration of THA was calculated from the peak height using a standard curve.

RESULTS AND DISCUSSION

Representative chromatograms of rat plasma and brain samples are shown in Figs. 1 and 2 utilizing optimal chromatographic conditions. No interfering peaks were seen in control samples of rat plasma (Fig. 1A) and brain (Fig. 2A). The following drugs which may be administered concurrently with THA injection, did not interfere with the assay: L-dopa, guanidine, haloperidol, apomorphine, oxotremorine, physostigmine, and probenecid. Standard curves for THA in rat plasma and brain were linear over the concentration range 0.1—10 μg/ml or g, respectively. Over this concentration range, the within-day and day-to-day precision (R.S.D.) values were 3.8% (n = 15) and 6.9% (n = 15), respectively. The overall recovery of THA added to rat plasma per ml and brain per g with concentrations between 0.1 and 10 μg was 53 ± 3.6% (mean ± S.D., n = 25). The recovery could be increased to 70% when 1 ml of methanol was

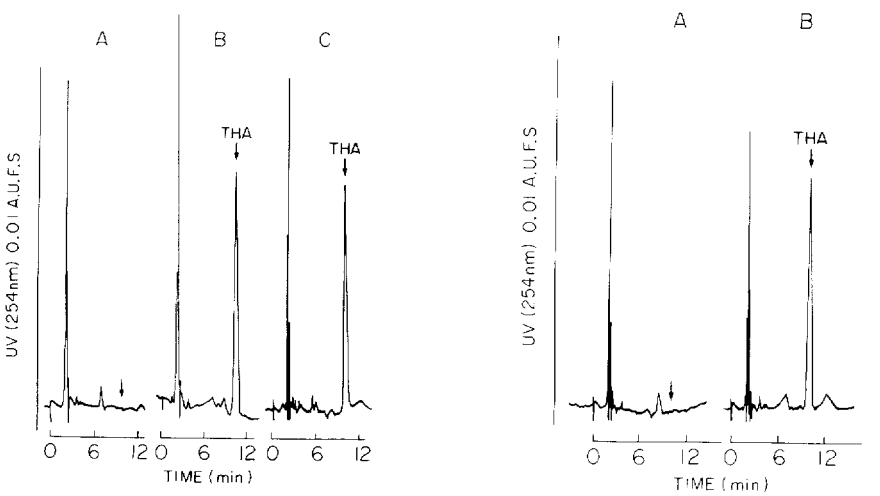

Fig. 1. Chromatograms of (A) blank rat plasma; (B) rat plasma containing 3.0 μg/ml of THA; and (C) human plasma containing 2.8 μg/ml of THA. Conditions were as given in the Experimental section.

Fig. 2. Chromatograms of (A) blank rat brain; and (B) brain containing 1.9 μg/g of THA. Conditions were as given in the Experimental section.

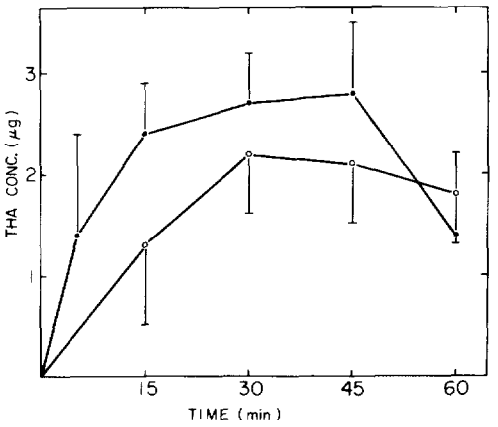

Fig. 3. Time—concentration curves for rat plasma in μg/ml (●——●) and brain in μg/g (○——○). Each point represents the mean value ± S.D. of five rats.

passed through the cartridge. However, under these circumstances, increasing noise was observed. The detection limit of THA was 100 ng/ml of plasma.

The addition of an ion-pairing reagent e.g. sodium dodecyl sulfate to rat plasma samples did not affect the recovery of THA. This behavior is unlike that of physostigmine in our previous report [10]. It is crucial to evacuate the cartridge before adding the washing fraction (0.3 ml) of methanol otherwise low recovery (< 30%) will be obtained.

The method described was applied to the quantitation of a series of rat plasma and brain samples obtained after intraperitoneal administration of THA (20 mg/kg). The time course of THA concentration in rat plasma and brain is illustrated in Fig. 3. The plasma curve reveals that the absorption of THA is very rapid and peak concentrations (2.4—2.6 μg/ml) are reached with 30—45 min. THA seems to cross the blood—brain barrier readily as shown in Fig. 3 and peak brain levels occur by 30 min after injection.

Human plasma samples spiked with various amounts of THA were also assayed. Fig. 1C shows a representative chromatogram of human plasma. No endogenous compounds of human plasma interfered with the detection of THA. Linearity between the detector response and the concentration of THA added was observed over the range of 0.1—20 μg. Precision and recovery data were similar to those of rat plasma and brain. This method is currently being utilized in pharmacokinetic studies in psychiatric patients receiving THA, and correlations of plasma concentrations with therapeutic response will be investigated.

The purification procedure using C_{18} Sep-Pak cartridge was found to be superior to the conventional, two-step extraction procedure [9]. It has the advantages of convenience, enhanced recovery and reproducibility, is less time-consuming, and more economical. The cartridge can be used at least three times without losing its isolation efficiency. One technician can easily prepare 60 samples per day.

Finally, the following compounds have been isolated, in our laboratory, utilizing this purification and enrichment method from the biological matrixes

including: physostigmine, probenecid, catecholamine and metabolites, and various polypeptides [12].

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