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Title: Rapid Analysis of Trifluoroacetic Acid and Bromide

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Introduction: Trifluoroacetic acid (TFA) is a volatile end product of the metabolism of halothane, isoflurane, and fluroxene, and bromide is an end product of the metabolism of halothane. Analytical methods for the determination of TFA (1,2) and bromide (3,4) in biological materials include a variety of gas chromatographic (GC) methods which require extractions or complicated derivatization techniques. We have developed a simple, sensitive procedure for the stimultaneous analysis of TFA and bromide for use in in vivo pharmacological studies in which the methyl ester of TFA (MTFA) and methyl bromide (MB) are quantified by a head-space GC technique with flame ionization detection (FID).

Methods: Aliquots (100-200 µl) of urine, plasma or whole blood were pipetted into 1 ml screw cap microreaction vials with PTFE lined septums containing 500 µl of cold H_2SO4. After addition of the methylating agent, dimethylsulphate (100 µl), the vials were sealed, mixed, and heated at 60°C for 20 min. After equilibration at 30°C for 20 min, 1 ml of head-space vapor was drawn slowly into a gas syringe, and injected onto the GC for MB and MTFA separation and detection. A Varian 3700 GC equipped with an FID, and a 1.8 m x 2 mm nickel column containing Porapak Q was used. The injection port was maintained at 130°, the column at 100°, and the detector at 150°C. The identities of the metabolites were confirmed with a 3300 Finnigan GC-mass spectrometer. Prior to analysis the samples, vials, sulphuric acid, and methylating agent were placed on ice for 45 min. A stock solution of TFA and NaBr was prepared in distilled deionized water. Working standards were prepared by adding aliquots of serial dilutions of the stock solution into vials containing plasma, whole blood, or urine and then treated as above. Sample TFA and bromide concentration were calculated from linear regression calibration curves. Male Sprague-Dawley rats were exposed to halothane (1%) for two hours. Urine and blood samples were taken at various times before and after exposure. Urine and blood samples were also obtained from consenting adult patients undergoing surgery with halothane anesthesia. The urine and blood samples from both rats and humans were stored frozen until analyzed.

Results: The head-space injection from a derivatized 24 hour urine sample from a halothane anesthetized rat had baseline resolution of MB and MTFA from normal urine constituents with an analysis time of 15 minutes per injection. Chromatograms of plasma and whole blood samples yielded very similar separation and resolution. A peak with retention time of 6.3 minutes (MB) corresponded to sodium bromide treated standards and a peak at 12.2 minutes co-chromatographed with authentic MTFA. A linear relationship between detector response of the head-space vapors of MTFA and MB and concentration of TFA and bromide in the liquid phase exists within the concentration range studied (.02-10 mM). Correlation coefficients were greater than 0.990. The standard concentrations of TFA and bromide reflect the experimental range of central venous blood concentrations in rats and humans exposed to halothane and urine concentrations in rats exposed to halothane under various conditions. TFA detection limits were approximately 2.5 µM for 200 µl plasma and 10 µM for 100 µl urine. Detection limits for bromide in blood and urine were reflected by background levels found in samples from rats and humans not exposed to halothane. A bromide concentration of 25 µM above background could be accurately determined. The method is reproducible with a 2.4% relative standard deviation (RSD) for TFA and 1.9% RSD for bromide for triplicate analyses. Comparison of the GC bromide assay with a modified bromide rosaniline colorimetric assay (5) gave good agreement between the two assays. Urine from consenting adult patients receiving halothane was analyzed for MTFA and MB during and after anesthesia. The values obtained are in good agreement with existing excretion rates determined by other methods.

Discussion: We have presented a rapid, simple technique for the analysis of TFA and bromide in biological samples obtained from rats and humans. The technique is reproducible and sensitive to the concentration range normally expected during and following anesthesia. This method will now allow us to rapidly and accurately study the oxidative metabolism of halothane and other anesthetics producing these metabolites. (Supported in part by NIH Grants AM 16715-07 and CA 21820-03).

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