Title : IDENTIFICATION OF SITES OF CELLULAR RNA SYNTHESIS WHICH ARE SENSITIVE TO HALOTHANE EXPOSURE

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Introduction. It is generally accepted that halothane exposure does not affect most cellular RNA synthesis since the total amounts of acid precipitable material[1] or the number of bands seen by polyacrylamide gel electrophoresis[2] following cell disruption are approximately the same following prolonged anesthetic exposure as without. However, the methods described above cannot distinguish whether all RNA species are synthesized at near normal levels during anesthetic exposure, or if a small number of species are dramatically inhibited. This becomes an important consideration when one realizes that only certain sites within the cell are affected during anesthetic exposure, and that RNAs which are transcribed, translated and/or processed may be selectively affected depending upon where in the cell they are metabolized or the physiological state of the cell. For example, the burst of RNA synthesis observed following the PHA-stimulation of human lymphocytes is reduced in the presence of 2.0% halothane.[3] Here we describe a technique which allows us to test whether individual species of RNA are synthesized during anesthetic exposure by taking advantage of the ability of RNA molecules to specifically hybridize to DNAs with complementary sequences.

Methods. To determine how much of a given RNA species exists in the cell, three things must be done. First, the RNA to be tested must be purified. Secondly, a radiolabeled DNA copy (or probe) must be biochemically synthesized. Thirdly, this probe must be reacted with a crude cell extract of nucleic acids. Under the appropriate conditions molecular hybridization will occur if nucleic acid sequences complementary to the probe are present and the rate of the hybridization reaction is directly proportional to the amount of the given species of RNA present in the cell. For these experiments, highly purified RNAs were isolated from monkey kidney (Vero) cells following swelling in hypotonic buffer and lysis in a Dounce homogenizer. Cell debris was removed by differential centrifugation (800 x g; 10 min) and the RNAs were isolated in crude form by rate zonal centrifugation in sucrose gradients. The RNA-containing fractions were dialyzed and treated with sodium dodecyl sulfate (1%) and proteinase K (100ug/ul) for 20 min (65°C; pH 8.3) to remove contaminating proteins, twice extracted with phenol and chloroform and precipitated from solution in ethanol containing 0.2M sodium acetate (pH 5.0). RNAs were then further purified by isopycnic sedimentation through CsCl. DNA copies of these RNAs were then made using reverse transcriptase employing ^{3}H-labeled thymidine and oligo calf thymus DNA as primer[4]. The RNA was eliminated by alkaline hydrolysis and the resulting complementary (c) DNA served as a probe for the presence of each cellular RNA species.

The amount of each RNA synthesized within the cell was assessed as follows. Crude cellular RNA was extracted from cells which had been exposed to various concentrations of halothane, employing the cell lysis and phenol extraction procedures described above. These crude RNAs were then incubated with a limited amount of radiolabeled cDNA at 69°C in .48M phosphate buffer (PB). At various times an aliquot of the reaction mixture was diluted to .14M PB and chromatographed on a hydroxyapatite (HA) column at 62°C. Under these conditions double-stranded (hybridized) materal sticks to the HA column in .14 PB while nonhybridized (single-stranded) material does not.

In these experiments the ribosomal (r-) RNAs of uninfected Vero cells as well as the viral RNAs of poliovirus and measles virus were measured by this technique in unexposed cells as well as cells exposed to 2.2% halothane.

Results. The amount of poliovirus RNA synthesized in the presence of 2.2% halothane was 100% that seen in unexposed poliovirus-infected Vero cells. However, the rate of annealing seen with cDNA directed against measles virus RNA sequences was only 5% of that seen in virus-infected cells not exposed to the anesthetic. A slight decrease in the amount of r-RNA was also observed. About 80% as much r-RNA was present per Vero cell following 30 hr of exposure to 2.2% halothane as compared to unexposed cultures.

Discussion. A different location within the cell is used for the synthesis of each of the three RNAs tested here. r-RNA is transcribed in the nucleus of the cell and is subsequently transported to the cytoplasm where it is processed (cleaved) and packaged with proteins into functional ribosomes. Poliovirus RNA is synthesized in the soluble cytoplasm of the cell using cellular enzymes, and it too is packaged with proteins to form a functional virus particle. Measles virus RNA is transcribed using a viral enzyme from within the virus core in the cellular cytoplasm where it is packaged with the measles virus structural nucleocapsid protein immediately following its synthesis. The differential sensitivity shown by these various RNAs to halothane exposure likely reflects an interaction between the anesthetic and sites at which the nucleic acids are synthesized or processed and suggests that halothane may affect various cellular sites to different degrees. It would be of interest to know whether RNAs which are transcribed, translated and/or processed in cellular membranes (such as vesicles or the endoplasmic reticulum) or other specialized compartments of the cell are as sensitive to anesthetic exposure as those which are metabolized in the cytoplasm. It is hoped that this technique will allow us to explore specific sites within the cell in order to determine which of these may be the target site(s) during anesthetic exposure.

References.

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