Vol. 120, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 16, 1984 Pages 150-155

INDUCTION OF A 'STRESS' PROTEIN IN INTACT MAMMALIAN ORGANS AFTER THE INTRAVENOUS ADMINISTRATION OF SODIUM ARSENITE

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Received March 1, 1984

Summary: The profile of nascent proteins synthesized in various rabbit organs after the intravenous injection of sodium arsenite was analyzed by the cell-free translation of purified polysomes. Examination of the translation products of polysomes isolated 1 hr after injection of sodium arsenite revealed a marked induction of synthesis of a protein of molecular weight 74,000 (74K) in the kidney, heart and liver which was similar to a 'heat shock' protein which was induced in these organs after elevation of body temperature by 2·5 to 3°C. Synthesis of the 74K protein was not detected in the translation products of brain polysomes isolated 1 hr after sodium arsenite injection.

Elevation of ambient temperature induces the synthesis of a set of 'heat shock' or 'stress' proteins in a wide range of tissue culture systems and unicellular organisms (1-3). This phenomenon is relevant to the intact mammal since increases in body temperature similar to that attained during fever reactions induce the synthesis of a 'heat shock' protein of molecular weight 70-74,000 in mammalian organs (4-12). Elevation of body temperature in rabbits by 2·5 to 3°C (i.e. hyperthermia) which is generated by either placement of animals at increased ambient temperature or intravenous injection of the psychotropic drug LSD, rapidly induces synthesis of a 74K protein in the brain, heart and kidney of the adult and fetal rabbit (11,12).

The objective of the present report is to investigate whether the synthesis of a 'stress' protein can be induced in intact organs of the rabbit by the intravenous injection of a toxic agent such as sodium arsenite which does not influence body temperature at the dosage used. Arsenic compounds are environmental pollutants which can orginate from the application of pesticides and herbicides or through emissions from coal-fired or geothermal industrial plants or from the smelting and processing of non-ferrous metals (13). These industrial

0006-291X/84 $1.50

activities can generate trivalent arsenite compounds which are more toxic than the pentavalent arsenates.

MATERIALS AND METHODS

Administration of sodium arsenite and induction of hyperthermia: Sodium arsenite dissolved in 0·9% NaCl was injected intravenously into 4 kg male New Zealand white rabbits at 0.8 mg/kg body weight and animals were sacrificed after 1 hr. Controls received saline injections. Elevation of body temperature in rabbits was induced by the intravenous injection of d-lysergic acid diethylamide (LSD) at 100 ug/kg body weight as previously described (11). Colonic temperature was continously monitored with a rectal thermistor probe. Animals which were injected with LSD demonstrated an increase in body temperature from 39.6 ± 0·2°C to 42·5 ± 0·3°C, 1 hr after drug administration. No change in body temperature was observed in animals which received sodium arsenite injections.

Analysis of cell-free translation products of isolated polysomes: Free polysomes were isolated from kidney, liver, heart and the cerebral hemispheres of the rabbit brain and subsequently translated in a micrococcal nuclease-treated reticulocyte lysate system with[^{35}S]methionine as previously described (5,11). Equal amounts of acid precipitable radioactivity (500,000 cpm) from the cell-free translation products of polysomes isolated from sodium arsenite treated, hyperthermic and saline injected control animals were than analyzed by two-dimensional polyacrylamide gel electrophoresis and fluorography (14, 15). The first dimension (horizontal axis) consisted of isoelectric focusing in a pH gradient (pH 5.5-8) followed by 7-17% gradient sodium dodecyl sulfate polyacrylamide gel electrophoresis in the second dimension. Data representative of at least three trial experiments are shown.

RESULTS

The profile of nascent proteins synthesized in various rabbit organs was examined by translating purified polysomes in an mRNA-dependent reticulocyte lysate system in the presence of[^{35}S]methionine followed by two-dimensional gel electrophoresis and fluorography. The translation products of kidney polysomes isolated from saline injected control animals are seen in Fig. 1a. The prominent induction of synthesis of a heat shock protein of molecular weight 74,000 (74K) (encircled in Fig. 1b) is readily apparent in the translation products of kidney polysomes which were isolated following hyperthermia induced by the intravenous injection of the psychotropic drug LSD which rapidly increased body temperature by 2·5 to 3°C. In previous studies we have demonstrated that hyperthermia induced by this drug stimulates the synthesis of a 74K heat shock protein in all organs of the rabbit which were examined (4,5,7,8,11). Blockage of drug-induced hyperthermia prevents the induction of synthesis of this heat shock protein (9,11).

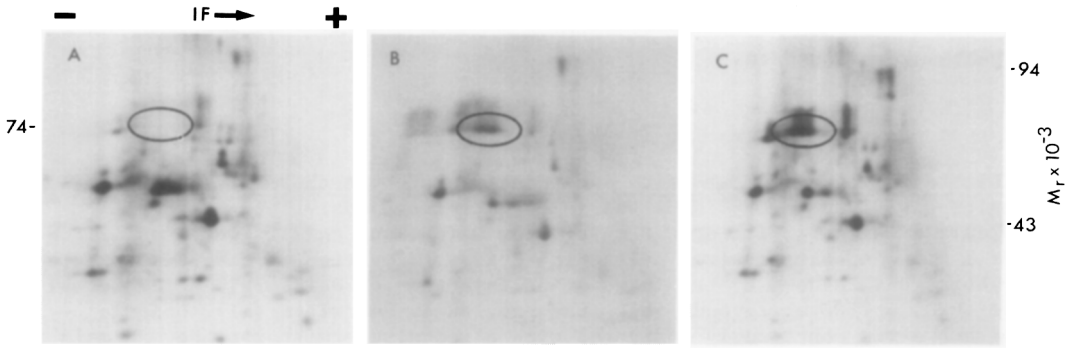

Fig.1: Cell-free translation of kidney polysomes after the intravenous administration of sodium arsenite or drug-induced hyperthermia. The position of the 74K 'stress' protein is encircled. a) Saline injected control; b) drug-induced hyperthermia; c) sodium arsenite treatment. In the presence of [^{35}S] methionine a mRNA-dependent reticulocyte lysate was programed with free polysomes isolated from rabbit kidney 1 hr after either the intravenous injection of sodium arsenite or drug-induced hyperthermia as described in Materials and Methods. No change in body temperature was detected after sodium arsenite treatment. Equal amounts of acid precipitable radioactivity (500,000 cpm) were analyzed by two-dimensional gel electrophoresis and fluorography. IF, isoelectric focusing.

When kidney polysomes were isolated from animals 1 hr after the intravenous administration of sodium arsenite, analysis of the labeled translation products revealed the marked induction of synthesis of a 74K protein (Fig. 1c). The body temperature of the rabbits, which is normally 39·6 ± 0·2°C, did not change following the injection of sodium arsenite at a dosage of 0·8 mg/kg body weight. Since sodium arsenite did not induce an increase in body temperature the 74K protein is more appropriately termed a 'stress' protein rather than a 'heat shock' protein.

Sodium arsenite also induced the synthesis in the heart and liver of a 74K protein which was similar in molecular weight to the heat shock protein which was induced in these organs following elevation of body temperature (Fig. 2). The appearance of mRNA coding for the 74K protein in polysomes was transient since polysomes isolated 24 hr after injection of sodium arsenite showed no evidence of the synthesis of the 74K protein when translation products were examined (data identical to Fig. 1a and 2a).

Tissue-specific differences in the inducibility of the 74K stress protein were apparent since injection of sodium arsenite into rabbits did not induce synthesis of this protein when translation products of brain polysomes were

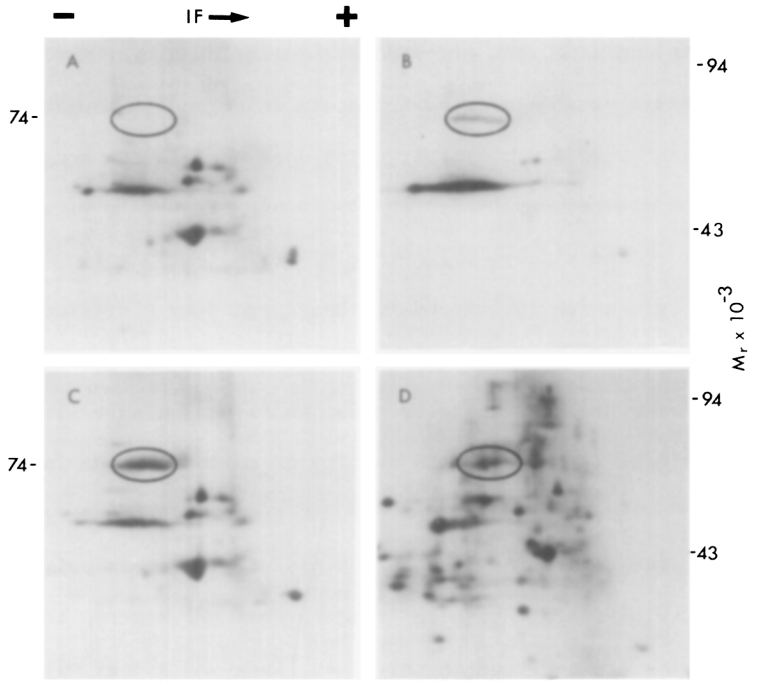

Fig.2: Induction of a 74K 'stress' protein by heart and liver polysomes. Heart- a) saline injected control; b) drug-induced hyperthermia; c) sodium arsenite treatment. Liver- d) sodium arsenite treatment. After injection of sodium arsenite or drug-induced hyperthermia as described in Fig.1, polysomes were isolated from heart and liver and translated in a reticulocyte cell-free system. The [^{35}S]methionine labeled translation products were then analyzed by gel electrophoresis and fluorography. The position of the 74K stress protein is encircled. IF, isoelectric focusing.

examined (Fig. 3a and c). Following hyperthermia, synthesis of the 74K protein was apparent when the translation products of brain polysomes were analyzed (Fig. 3b).

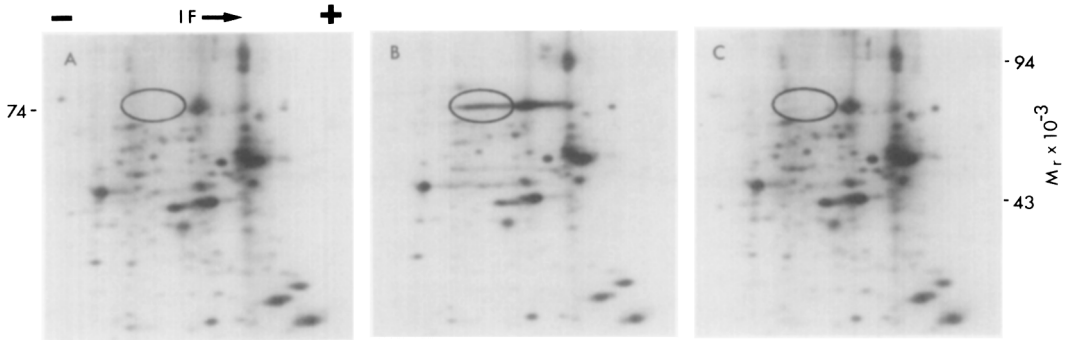

Fig.3: Cell-free translation of brain polysomes. a) Saline injected control; b) drug-induced hyperthermia; c) sodium arsenite treatment. The 74K stress protein is encircled. IF, isoelectric focusing.

DISCUSSION

These results indicate that perturbations other than hyperthermia can induce the synthesis of a major stress protein in organs of the intact mammal. A toxic agent such as sodium arsenite, which does not elevate body temperature in rabbits at the dosages employed, rapidly induces synthesis of a 74K protein in the heart, liver and kidney. This protein is similar in molecular weight to a heat shock protein which is induced in these organs by elevation of body temperature. It has been noted that sodium arsenite can induce synthesis of heat shock or stress proteins in cells grown in tissue culture (16-19).

Organ-specific differences in the ability of an intravenous injection of sodium arsenite to induce synthesis of the 74K protein were apparent since this protein was not detected in the translation products of brain polysomes. Studies on the organ distribution of arsenite indicate that the highest tissue concentrations in rabbits are observed in the liver, lung and kidney with a comparatively low accumulation in the brain (20,21). A recent study in monkeys suggests that sodium arsenite rapidly accumulates in the liver and kidney and that there is a very slow passage of the compound through the blood-brain barrier into the brain (22).

Translation of purified polysomes is an effective method to examine the profile of nascent proteins which are synthesized in various mammalian organs after perturbation treatments since more highly labeled nascent proteins can be obtained than is possible by *in vivo* injection of labeled amino acids and difficulties arising from possible perturbation-induced changes in the rate of uptake of labeled amino acids or in amino acid pool sizes are avoided.

ACKNOWLEDGEMENTS

This project was supported by grants to I.R.B. from the Medical Research Council of Canada.

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