REVERSIBLE ALTERATIONS IN MEMBRANE PERMEABILITY OF *ESCHERICHIA COLI* INDUCED BY A STEROIDAL DIAMINE, IREHDIAMINE A

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Mahler and Baylor (1967) introduced steroidal diamines such as irehdiamine A (IDA) as a class of potentially useful agents with selective inhibitory effects on bacterial and bacteriophage growth. In bacteriophage-infected cells, phage-directed DNA synthesis is more sensitive to IDA inhibition than is RNA and protein synthesis (Mahler and Baylor, 1967). *In vitro* associations of IDA and polynucleotides have been extensively studied (Mahler *et al.*, 1966; Goutarel *et al.*, 1967; Lefresne *et al.*, 1967).

We have recently reported rapid and selective effects of steroidal diamines on cell membrane properties which we proposed as an explanation for the inhibitory effects of the steroids (Silver and Levine, 1968). The initial changes in cell permeability properties and active transport, which are induced by IDA within a minute or so of the time of addition, were found to be reversible. In this paper, we describe the reversal of the effects of IDA by dilution and by high concentrations of magnesium.

Material and Methods

Effects of steroidal diamines on membrane permeability properties of *E. coli* strain B were measured by following both the influx (by active transport mechanisms) and the efflux (leakage) of radioactive ^{42}K and ^{14}C-thiomethylgalactoside (TMG) as previously described in greater detail (Silver and Levine, 1968; Silver and Wendt, 1967). These two very different materials were used to test the generality of the effects which IDA induces. Although the ^{42}K experiments

were carried out at 25° C and the ^{14}C-TMG experiments at 4° C because of the differences in the inherent rate constants for the two systems, each type of experiment described has been performed with both systems with similar results.

Results and Discussion

Although the inhibitory effects of IDA on potassium influx can be observed within one or two minutes at 25° C, the initial inhibition is reversible. Irreversible inhibition of the potassium active transport system occurs with a half-life of between 10 and 30 minutes depending upon conditions of recovery and concentration of IDA (Fig. 1; Silver and Levine, 1968).

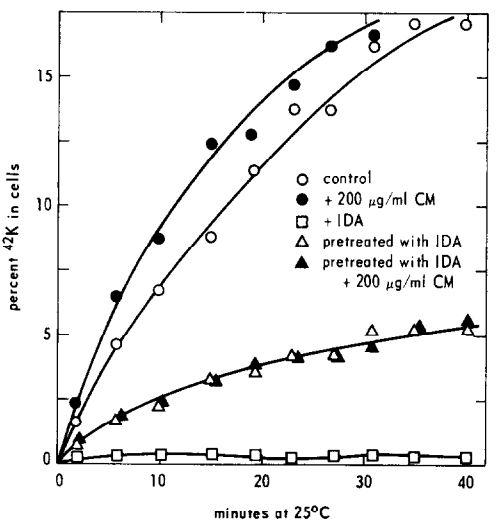

Fig. 1. Recovery of potassium active transport in the presence of chloramphenicol. Control cultures and cultures pretreated for 15 minutes with 10^{-4} M IDA were centrifuged and resuspended with or without chloramphenicol. IDA or chloramphenicol was added to control cultures 1 minute before the addition of ^{42}K. ^{42}K was added and influx measured with washed millipore filtered samples.

The measurable recovery of the ability to concentrate potassium shown in Fig. 1 allows us to ask (1) whether the reversibility of IDA inhibition is dependent upon cellular metabolism and resynthesis of material removed or destroyed by IDA or (2) whether the recovery reflects a structural, conformational change in the membrane induced by IDA and which spontaneously reverts when the IDA is removed (as by analogy to a "self-sealing" inner tube when a nail is removed).

The energy dependence of the reversible effects on active transport cannot be measured since active transport is itself energy-dependent. However, the reversal of the inhibition of potassium influx after the removal of IDA does not require protein synthesis (Fig. 1). In this experiment, 200 µg/ml of the protein synthesis inhibitor, chloramphenicol, was added to the IDA pretreated

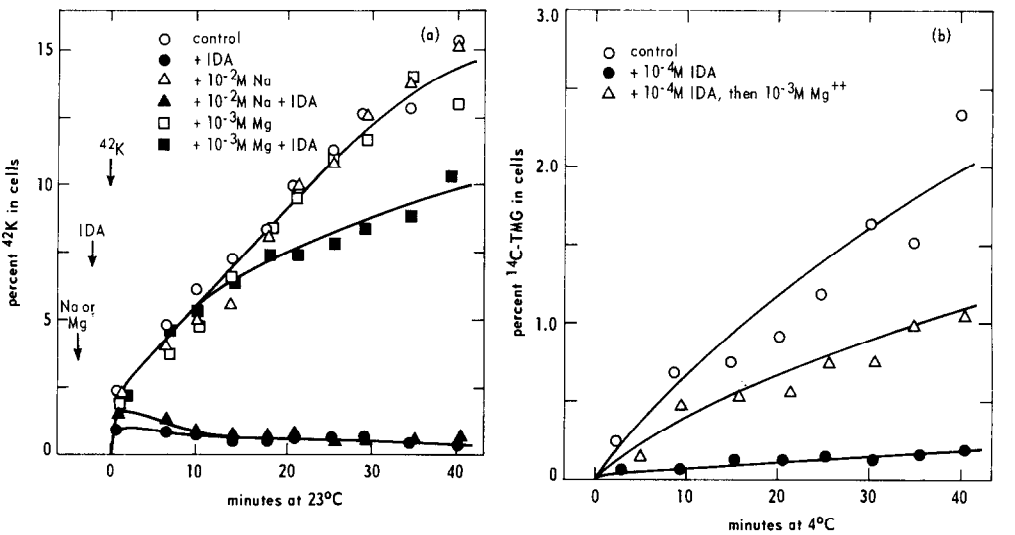

Fig. 2. Effects of magnesium and sodium on IDA inhibition of potassium and TMG active transport. (a) 10^{-3} M $MgCl_2$ or 10^{-2} M NaCl was added 3 minutes before and 10^{-4} M IDA 2 minutes before the addition of ^{42}K. (b) 10^{-4} M IDA or IDA immediately followed by 10^{-3} M $MgCl_2$ was added 2 minutes before the addition of ^{14}C-TMG.

cells just prior to the centrifugation to remove the IDA and in the IDA-free resuspension medium. The presence or absence of chloramphenicol was without detectable effect on the recovery. The level of recovery in this experiment was about 30 percent after 15 minutes of pretreatment.

The 14C-TMG active transport system is reversibly inhibited by IDA in a fashion quite similar to the potassium system. ^{14}C-TMG influx has been measured at both 4° C and 25° C; the IDA inhibition was much more readily reversible at 25° C than at 4° C (unpublished data). We have not pursued the physical basis for this temperature effect, but it is possible that it reflects a temperature dependent change in the structure of the cell membrane (e.g. Silver et al., 1968) so that the membrane is able to release IDA and/or resume its original conforma-

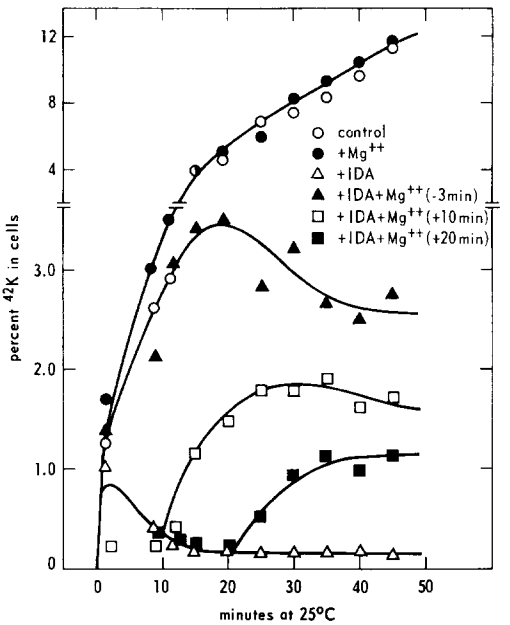

Fig. 3. Reversal of IDA inhibition by magnesium. To control cultures and cultures inhibited with 10^{-4} M IDA (added 2 minutes before the ^{42}K), 10^{-3} M $MgCl_2$ was added as indicated.

tional state only at the elevated temperature.

The effects of IDA on bacteriophage growth, active transport and cell permeability, and nucleic acid structure are all ionic strength dependent (Mahler et al., 1966; Mahler and Baylor, 1967; Lefresne et al., 1967; Silver and Levine, 1968) but the physical basis for this is not clear. The experiments in Fig. 2 show that high magnesium concentrations prevent or limit the effectiveness of IDA in inhibiting potassium and TMG influx. Note that although magnesium competes with IDA, sodium does not (Fig. 2 a). (IDA is itself a divalent cation; other cations have not been tested in this system.) When high magnesium concentrations are added to IDA-inhibited cells 10 or 20 minutes after the addition of IDA, a partial reversal of the IDA-inhibition of potassium influx is seen (Fig. 3) and the extent of this reversal depends on the length of the delay between the addition of IDA and the addition of magnesium. The data again show an irreversible inactivation of potassium active transport with a half-life of about 10-15 minutes at 25° C. In the comparable experiments with ^{14}C-TMG, the addition of

10^{-3} M $MgCl_2$ before or immediately after the addition of IDA greatly reduced the IDA inhibition of TMG influx (Fig. 2 b). But the addition of $MgCl_2$ 10 or 20

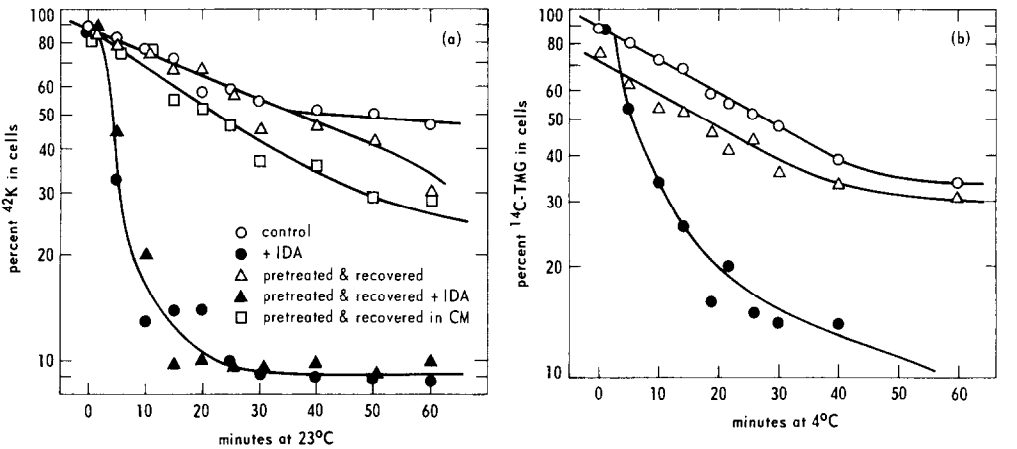

Fig. 4. Recovery of efflux rates for ^{42}K and ^{14}C-TMG. Controls or cells pretreated with 10^{-4} M IDA and "loaded" with ^{42}K and ^{14}C-TMG during 15 minute recovery periods were resuspended in fresh media and efflux of radioactivity followed by millipore filtration.

minutes after the addition of IDA did not lead to a recovery of influx. This may be related to the temperature effect on the reversal of IDA inhibition by dilution referred to above.

In addition to limiting the influx of materials into the cell by active transport, IDA greatly increases the rate of efflux (leakage) of small molecules from treated E. coli (Silver and Levine, 1968). The cells which recover the ability to concentrate potassium or TMG must recover the relatively impermeable state of the untreated cells in order to accumulate measurable amounts of potassium or TMG. If the cells remained "leaky", even though the active transport systems were active, the cells would lose the materials as fast as they accumulated them. The recovery of the relatively impermeable state was measured directly in the experiments described in Fig. 4. In these experiments, the bacteria, either control cultures or pretreated with IDA, were "loaded" with radioactive potassium or TMG, resuspended in fresh media, and the efflux (leakage) of radioactivity from the cells was measured. The rates of efflux

from pretreated cells were very similar to those from the untreated control cells. This recovery to a relatively impermeable state occurs even in the presence of chloramphenicol (Fig. 4 a).

In summary, we have measured reversible effects of the steroidal diamine IDA on the influx and efflux of radioactive potassium and TMG in treated E. coli. The recovery to the original state with active transport and relatively slow efflux does not require protein synthesis.

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