Regulation of Ornithine Decarboxylase Expression by Anisosmotic Shock in α-Difluoromethylornithine-resistant L1210 Cells*

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Ornithine decarboxylase (ODC) activity is known to be strongly enhanced in mammalian cells by a sudden reduction in ambient osmolality. The effect of hyposmotic shock on the regulation of ODC protein and mRNA levels was studied in a variant L1210 mouse leukemia cell line (D-R cells) which expresses ODC at ≥100-fold higher levels than the parental cells. Hyposmotic stress increased ODC activity in proportion with the osmotic gradient imposed to both D-R cells and their normal counterparts. A 60% decrease in medium osmolality increased ODC activity and the amount of immunoreactive ODC protein from 20- to 30-fold after 4 h without any detectable change in ODC mRNA contents in D-R cells. ODC induction was sustained up to 48 h after hyposmotic shock, with maximal activity levels being observed at 24 h. Hypotonic shock dramatically increased (up to 36-fold) the rate of ODC synthesis as measured by 10-min pulses with [35S]methionine, in agreement with kinetic constants predicted from the changes observed for the enzyme activity. Moreover, hyposmotic stress extended the half-life of ODC activity from 35 ± 10 to 212 ± 67 min and blocked any degradation of the radiolabeled immunoreactive protein, which had a half-life of 28 ± 6 min under isotonic conditions, for at least 120 min after addition of cycloheximide. The induction of ODC by hyposmotic stress was quickly reversed by a sudden upshift of osmolality through a very rapid inhibition of ODC biosynthesis and an increase in the rate of enzyme degradation. Thus, hyposmotic stress activates the expression of ODC exclusively through post-transcriptional mechanisms in D-R cells. The osmotically induced accumulation of ODC molecules is quite unique as shown by the fact that ODC is the major protein (∼25% of total) synthesized during the first 4 h following a 60% hypotonic shock, despite a 30–50% reduction of the rate of labeled precursor incorporation into soluble proteins.

It has repeatedly been observed that the expression of ODC is the major protein (∼25% of total) synthesized during the first 4 h following a 60% hypotonic shock, despite a 30–50% reduction of the rate of labeled precursor incorporation into soluble proteins.

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EXPERIMENTAL PROCEDURES

Materials—1-[35S]Methionine (1000–1200 Ci/mmol), cytidine 5'-[α-32P]triphosphate (>800 Ci/mmol) and cytidine 5'-α-[35S]thiotri-

1 The abbreviations used are: ODC, ornithine decarboxylase (EC 4.1.1.17); DFMO, α-difluoromethylornithine; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mops, 3-(N-morpholino)propanesulfonic acid.

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Ornithine was purchased from Du Pont-New England Nuclear. RPMI 1640 medium was reconstituted from the individual components in order to allow independent variations of the NaCl concentration. Amino acids and vitamins used for the fabrication of modified RPMI 1640 media were from Sigma and GIBCO Laboratories, respectively. The plasmid pmODC-1 containing a mouse ODC cDNA was a gift provided by Drs. D. Nathans and Dr. C. Kahana (11). (The pGEM3zf(−)-ODC was constructed by inserting the ODC cDNA insert from pmODC-1 into the multiple cloning site of pGEM3zf(−) obtained from Promega (Madison, WI). SP6 polymerase and EcoRI were purchased from Bethesda Research Laboratories. Other biochemical reagents were of analytical grade and obtained from Sigma, Bio-Rad, ICN BioMedicals, and Fisher.

**Cell Culture Conditions**—L1210 mouse leukemia cells were grown as suspension cultures in RPMI 1640 medium supplemented with 10% Nu-Serum (Collaborative Research Inc., Lexington, MA), 16 mM Hepes, 7.8 mM Mops, 2 mM L-glutamine, and antibiotics as described (12) in an atmosphere of 5% CO2, 95% air at 95% relative humidity. The DFMO-resistant (D-R) subline derived from the L1210 cell line (9) was routinely grown in the same medium in the additional presence of 10 mM DFMO. Prior to osmotic shock experiments, 3- to 4-day-old D-R cell cultures were washed free of DFMO, seeded at 1 × 10^6 cells/ml, and grown for 60 h in the absence of the drug.

**Osmotic Shock Experiments and Metabolic Labeling with [35S]Methionine**—Cells grown in DFMO-free medium were harvested by low-speed centrifugation and resuspended in serum-free RPMI 1640 medium supplemented with 10% Nu-Serum (Collaborative Research Inc., Lexington, MA), 16 mM Hepes, 7.8 mM Mops, 2 mM L-glutamine, and antibiotics as described (12) at 37 °C and equilibrated with 5% CO2 (10% relative humidity). The DFMO-resistant (D-R) subline derived from the L1210 cell line (9) was routinely grown in the same medium in the additional presence of 10 mM DFMO. Prior to osmotic shock experiments, 3- to 4-day-old D-R cell cultures were washed free of DFMO, seeded at 1 × 10^6 cells/ml, and grown for 60 h in the absence of the drug.

**Effect of Hypoosmotic Stress on ODC Activity**—In all experiments described in the present study, serum, a well-known inducer of ODC (22), was omitted from the medium formulation to avoid repeated centrifugation of the cell suspensions necessary for subsequent protein analysis, since cells were found to become exceedingly fragile under hypoosmotic conditions. However, other medium components known to modulate ODC activity independently of the external osmolality such as amino acids using the A transport system (6, 23-26) and glucose (27) were kept constant, the osmolality being manipulated by varying only the NaCl concentration.

The effect of hypotonic shock on ODC activity in L1210 cells and their DFMO-resistant counterparts was examined following a 4-h incubation in RPMI 1640 medium with osmolality adjusted from 130 to 325 mosm/kg with NaCl. As shown in Fig. 1, the level of ODC activity was inversely correlated with the osmolality in both L1210 and D-R cells. Thus, as osmolality was reduced from 325 to 130 mosm/kg, ODC activity measured at 4 h was increased by a factor of up to 40- and 20-fold in L1210 and D-R cells, respectively. The effect of diluting the medium with water to produce an identical decrease in osmolality yielded qualitatively similar results, although the induction was only about 5-fold in both cell lines (data not shown).

The time course of the induction of ODC activity by a 60% hypotonic shock in D-R cells is presented in Fig. 2. The most rapid phase of induction of enzyme activity occurred in the first 4 h and resulted in a 9-fold increase over the initial ODC levels, with 50% of the increase being observed within 120 min following the onset of hypotonic stress. The differential effect of hypotonic treatment was, however, even larger (about 30-fold), because ODC activity decreased exponentially...
by about 70% during that period in cells incubated under isotonic conditions. This decrease in enzyme activity observed in control cells was prevented by reintroducing the serum component (data not shown). Following this initial period of rapid induction, hypoosmotic conditions further sustained a slower increase in ODC activity up to about 24 h after the downshock. The enzyme activity (40,637 ± 937 units/mg) measured at this stage represented a 270-fold difference with isosmotic conditions and a 17-fold increase over the initial ODC activity levels. More prolonged incubation (up to 48 h) of D-R cells under serum-free conditions led to the appearance of gross vacuolation and cell lysis under both osmotic conditions. Nevertheless, ODC activity was still induced 5-fold over the initial levels in D-R cells incubated for 48 h at 130 mosm/k. Furthermore, a sudden reversal of osmolality from 130 to 325 mosm/kg (Fig. 2, arrow) induced a rapid decrease in ODC activity in cells incubated previously for 2 h under hypoosmotic conditions. Enzyme activity levels had reached control values 6 h following the hypertonic shock. Likewise, ODC activity decayed even more rapidly in cells maintained for 2 h in isotonic medium and then exposed to an increase in osmolality of the same absolute magnitude (from 325 to 520 mosm/kg).

The rate of incorporation of L-[35S]methionine into immunoreactive ODC was also dramatically increased in D-R cells incubated under hypoosmotic conditions, with a pattern similar to that seen for the enzyme activity in the initial phase of the induction (Fig. 3). Radiolabeling of ODC typically reached a plateau after 2 h of incubation in cells subjected to hypotonic treatment, probably as a result of a general decrease in the rate of intracellular precursor incorporation (cf. Fig. 8A). Thus, the induction of ODC activity by hypotonic shock in D-R cells results from the rapid accumulation of de novo synthesized enzyme molecules. Taken together, the present results further indicate that the intracellular levels of enzymatically active ODC are rapidly modulated in a sustained but reversible manner by osmotic stress, the direction of the observed variations being inversely related to changes in medium osmolality.

Effect of Hypotonic Stress on ODC mRNA Levels—A 60% hypotonic shock did not result in any detectable change in mRNA content at the end of a 1- (results not shown) or 3-h incubation (Fig. 4), i.e. at periods of nearly maximal rate of increase in ODC activity levels (cf. Fig. 2). Thus, the increase in ODC activity induced by hypotonic shock in D-R cells is likely resulting from changes in the control of the enzyme expression at a post-transcriptional level.

Effect of Hypoosmotic Stress on the Rate of ODC Biosynthesis—The rate of ODC synthesis was determined during the rapid initial phase of induction of ODC by hypotonic shock by measuring immunoreactive ODC radioactivity at the end of 10-min pulses with L-[35S]methionine. Since the period of labeling was short as compared with the half-life of ODC under the present experimental conditions (cf. Figs 6 and 7), the rate of incorporation of the labeled amino acid likely reflected mostly the rate of de novo synthesis. Although the relative degree of increase of the rate of ODC synthesis measured by this short pulse method was found to differ between experiments, biosynthesis of ODC was indeed strongly accelerated by hypoosmotic shock, the differential effect being greater at earlier time points following transfer to low osmolality (Fig. 5A and Table I). A sudden reversal of osmolality from 130 to 325 mosm/kg decreased the apparent rate of enzyme synthesis to near control values within 10 min, whereas the transition from 325 to 520 mosm/kg practically abolished ODC synthesis (Fig. 5B). It must be noted, however,
that the rate of general incorporation of L-[35S]methionine into soluble proteins in these cells was even more severely inhibited by hypertonic (by 70 and 60% for cells initially at 325 and 130 mosm/kg, respectively) than hypotonic shock (29%) in these short pulse experiments. Nevertheless, strong indication that the high rate of ODC synthesis in hypotonically treated D-R cells is indeed drastically suppressed upon hypertonic shock comes from the fact that the reduced half-life of the enzyme under those conditions was barely affected by the addition of cycloheximide (vide infra). Thus, the effect of a decrease in the ambient osmolality on the rate of ODC biosynthesis is extremely rapid and, apparently, quickly reversible, thus reinforcing the notion that regulation of ODC expression by changes in osmolality is exerted at post-transcriptional steps.

Effect of Hypoosmotic Stress on ODC Degradation—The possible contribution of changes in the rate of ODC turnover in the accumulation of ODC in hypotonically treated D-R cells was investigated using two different approaches. First, cells were prelabeled for 15 min in isosmotic medium containing 0.16 μM of L-[35S]methionine as the sole source of the amino acid and then exposed to iso- or hypotonic conditions in the presence of 2 mM unlabeled L-methionine. Although radioactive methionine was not removed from the medium, such conditions closely approached those of a pulse-chase experiment as shown by the rapid disappearance (t½ = 31 min) of immunoreactive radiolabeled ODC in control cells (Fig. 6A). On the other hand, the decay of ODC labeling over
a 4-h period had a half-life of 164 min in cells transferred to
low osmolality medium, as measured by this method. How-
ever, the radioactivity of total soluble proteins also decreased
at a much slower rate in pulse-labeled cells transferred to 130
mosm/kg (data not shown).

Since the effect of hypotonic shock on apparent general
proteolysis suggested that the rate of dilution of the intracel-
lar free pool of L-[35S]methionine might be impaired under
hyposmotic conditions, a second type of measurement of
ODC degradation was obtained by exposing cells continuously
labeled with L-[35S]methionine to 200 μM cycloheximide. Par-
allel determination of the rate of ODC degradation with
cycloheximide yielded values of 106 ± 2 and 60 ± 6 min at
130 and 325 mosm/kg, respectively (mean ± S.D. of triplicate
determinations from two independent experiments) when the
drug was added at zero time of the incubation (Fig. 6B). On
the other hand, when cycloheximide was added 120 min after
osmotic shock, the intracellular content of radiolabeled ODC
(Fig. 7A) as well as ODC activity levels (Fig. 7B) were
remarkably stable. ODC activity had a half-life of 212 ± 67
min in seven independent cell incubations at 130 mosm/kg,
and extending the period of incubation with cycloheximide
up to 3 h did not affect the measured half-life of the enzyme
activity (t1/2 = 224 min). No parallel degradation of the im-
munoreactive protein was measurable at 130 mosm/kg up to
120 min after addition of cycloheximide. During the same
time interval, ODC had t1/2 values at 28 ± 6 min (n = two
experiments) and 35 ± 10 min (n = seven experiments) under
isosmotic conditions for the radiolabeled protein and enzy-
matic activity, respectively. A sudden hypertonic reversal to
normosmotic conditions accelerated the decay rate of ODC
activity and immunoreactive protein so that t1/2 was now 69
and 160 min, respectively, in cycloheximide-treated cells
preincubated for 2 h at 130 mosm/kg (Fig. 7, A and B). In
the absence of cycloheximide, ODC activity decayed with a half-
life of 73 min in cells submitted to reversal of hyposmotic
conditions (data not shown). Thus, the rapid decrease of ODC
activity in cells shifted from 130 to 325 mosm/kg was likely
caused by an acceleration of the degradation rate, in addition
to a strong inhibition of ODC biosynthesis. However, the
decay rate of ODC activity and immunoreactive labeled pro-
tein was not affected in cells shifted from 325 to 520 mosm/
kg.

These data thus suggest that a reversible stabilization of
ODC against proteolytic mechanisms is a major factor con-
tributing to its accumulation upon exposure of D-R cells to
hyposmotic shock. This decrease in ODC degradation rate
is more important at later stages in the induction of the
enzyme. Furthermore, an increase in ODC proteolysis also
plays an important role in the suppression of the enzyme
induction in cells exposed previously to hypotonic conditions
and shifted to normosmotic medium. However, an increase in
the degradation rate of ODC does not appear to participate,
at least at early stages, in the dramatic decrease of the enzyme
activity observed in cells subjected to a hypertonic shock
when previously kept under isosmotic conditions.

**Differential Effect of Hypoosmotic Stress on ODC Levels and**
FIG. 7. Late effect of hypoosmotic treatment on the rate of ODC degradation in D-R cells and its partial reversal by a sudden upshift of medium osmolality. A, cells were labeled for 2 h in serum-free RPMI 1640 medium at 325 (•) or 130 mosm/kg (○) containing L-[35S]methionine (19 μCi/ml) and then exposed to 200 μM cycloheximide for the indicated times prior to harvesting for immunoprecipitation and SDS-PAGE analysis of radiolabeled ODC. In parallel incubations, osmolality was shifted to either 520 (□) or 325 mosm/kg (▲) at the time of cycloheximide addition by supplementation of NaCl to cells preincubated at 325 and 130 mosm/kg, respectively. The fluorographs (A1) and densitometric analysis (A2) are shown for one representative experiment. B, enzyme activity measured under identical conditions. Points represent the mean ± S.D. of triplicate determinations from seven (○, □) or two (▲, △) independent experiments. Symbols are as in A.

General Protein Synthesis—As shown in Fig. 8A, the average rate of L-[35S]methionine incorporation into total soluble proteins was reduced by up to 50% by a 4-h exposure of D-R cells to hypoosmotic conditions. This inhibition became mainly apparent in the second half of the incubation period and was also observed when 10-min pulses with L-[35S]methionine were performed at different time points (data not shown). The fact that the total radioactive ODC content also reached a plateau after about 2 h of hypoosmotic incubation (cf. Fig. 3) despite a demonstrable stimulation of the rate of

FIG. 8. Effect of hypoosmotic stress on the incorporation of L-[35S]methionine into total soluble proteins and immunoreactive ODC in D-R cells. A, D-R cells were incubated with L-[35S]methionine (18 μCi/ml) at 325 (○, □) or 130 mosm/kg (●, ▲) and harvested at the indicated times. Cycloheximide was added (●, ▲) or omitted (○, □) 120 min after the onset of osmotic stress (arrow). Total 35S radioactivity incorporated into proteins present in ODC enzyme extracts was measured following trichloroacetic acid precipitation (10). B, cells were incubated for 4 h at 325 or 130 mosm/kg in the presence of L-[35S]methionine (19 μCi/ml) and then harvested for immunoprecipitation and/or SDS-PAGE analysis of ODC contents. Lanes 1 and 2 and 3 and 4, immunoprecipitated ODC from cells incubated at 325 and 130 mosm/kg, respectively; lanes 5–8, total proteins present in ODC enzyme extracts. Each lane corresponds to the analysis of 91 μg of total proteins present in the initial extract.

TABLE II

Comparison of the rates of ODC synthesis and degradation in D-R cells exposed to isotonic (325 mosm/kg) and hypotonic (130 mosm/kg) media, as based on enzyme activity measurements

The rate of synthesis (S) was calculated according to Berlin and Schimke (Ref. 28) using the expression $S = ([P/P_p] - e^{-kt}) \cdot (k'P_0)/(1 - e^{-kt})$, where $P_0$ is the initial ODC activity, $P_i$ the value of ODC activity measured after a time $t$, and $k'$, the fractional rate of ODC degradation. The kinetic parameters are calculated for the rates prevailing at the onset of the given interval. The values for $k'$ are derived from experiments presented in Figs. 6B and 7B, and the activity data ($P/P_p$) are collected from two to seven independent experiments. Other details are as given in the text.

| Osmolality Interval | $P/P_p$ | $10^3 \times k'$ | k$/P_p$ | S |
|---------------------|---------|----------------|---------|---------|
| mosm/kg             | h       | min⁻¹ | units/min/μg |
| 325 → 325           | 0 → 4   | 0.31 (n = 2) | 11.6 | 28 | 7.6 |
| 130 → 130           | 0 → 4   | 8.99 (n = 2) | 6.5 | 16 | 176 |
| 325 → 325           | 2 → 4   | 0.64 (n = 7) | 19.6 | 23 | 14 |
| 130 → 130           | 2 → 4   | 1.79 (n = 7) | 3.3 | 40 | 137 |
| 325 → 520           | 2 → 4   | 0.13 (n = 2) | 18.1 | 21 | 0.3 |
| 130 → 325           | 2 → 4   | 0.24 (n = 2) | 10.0 | 122 | <0.1 |

* Cells were subjected to the indicated osmotic shift by the addition of NaCl of the appropriate osmolality, following a 2-h period of incubation at the initial medium osmolality.
its biosynthesis at that stage (Fig. 5 and Table II) thus suggests that hypotonic treatment leads to a reduced pool of labeled methionine available for protein synthesis.

The densitometric pattern of cytosol proteins at the end of a 4-h period of labeling with L-[35S]methionine revealed a general decrease of about 30% in radiolabel incorporation with the exception of the ODC band, which represented 0.35 and 25% of total radiolabeled soluble proteins in control and hypotonically treated D-R cells, respectively (Fig. 8B). Thus, notwithstanding the fact that one-dimensional gel electrophoresis might leave minor proteins with similar properties undetected, these results indicate that the accumulation of ODC protein consequent to hypotonic shock appears to be quite unique and that ODC becomes the major protein synthesized in D-R cells under hyposmotic stress.

**Discussion**

The present study provides evidence that in an ODC-overproducing cell line, hyposmotic conditions induce a dramatic, sustained accumulation of enzymatically active ODC despite the presence of already high basal ODC levels. This increase is brought about by the combination of increased enzyme synthesis from a constant number of transcripts and decreased degradation of the enzyme. The lack of effect of hypotonic conditions on ODC mRNA content is consistent with the fact that actinomycin D did not affect the initial rate of induction of ODC activity elicited by hypotonic shock in other mammalian systems (2, 4). At the peak of enzyme activity induced by a 60% decrease in osmolality (~40 μmol/h/mg protein) in D-R cells, which is by far the highest level ever reported in a mammalian system, ODC represents about 1.3% of total soluble proteins, based on the estimated catalytic activity of homogenized mouse kidney ODC (13). Although decreasing the NaCl concentration to lower the osmolality may affect several aspects of the cellular response by changing the electrochemical gradients, the accumulation of ODC induced by that treatment most likely results from an osmotic effect. In experiments not presented here, ODC induction was abolished for at least 4 h when the osmolality of media with a reduced NaCl concentration was kept constant by the addition of “impermeant” osmolytes such as mannitol, sucrose, or choline chloride, as found in other mammalian systems (2–6).

The stimulatory effect of hypotonic stress on translational activity appears to be restricted to the expression of ODC protein. We cannot rule out the possibility that an increased rate of ODC biosynthesis might result from the sparing of ODC mRNA from a general inhibition of the translatability of other mRNAs, assuming that the basal rate of ODC mRNA translation is largely limited by its competition with other messages for a common step in protein biosynthesis such as polysome formation (29). However, the time-dependent decrease of the rate of L-[35S]methionine incorporation into ODC as well as total soluble protein induced by hypotonic stress strongly suggests that the rate of entry of the precursor into the pool available for protein synthesis was progressively impaired. Indeed, the incorporation of other amino acids such as glycine and leucine into trichloroacetic acid-insoluble material is also reduced by hypotonic stress in Ehrlich ascites tumor cells (30) and cultured mouse mammary glands (4). These effects are most likely resulting from the decrease in the influx rate and increase of the efflux rate of amino acids observed following hypotonic treatment (31). It is likely that such effects on the kinetics of methionine transport had minimal effects on the initial rate of ODC labeling in the short pulse periods used for its determination. Furthermore, any decrease in the transport rate of methionine would only underestimate the stimulation of the rate of ODC biosynthesis induced by hypoposmotic stress.

Measurements of the rate of ODC biosynthesis by the short pulse method are consistent with the kinetic analysis of changes in enzyme activity during the first 4 h of the time course (Table II). The rate of ODC degradation was seen to exceed the rate of its biosynthesis in cells kept under isotonic conditions, as expected from the gradual decay of the enzyme activity (Fig. 2). It is quite clear that the approximately 23-fold increase in the rate of ODC synthesis was the main factor responsible for the enzyme accumulation induced by hypotonic shock. The rate of ODC biosynthesis at 120 min was only slightly lower than that measured at the onset of hypoposmotic stress, indicating that the contribution of a reduction in the rate of ODC proteolysis was mostly important at later stages of the induction of ODC activity. Furthermore, the absolute rate of ODC synthesis became extremely low in cells shifted from 325 to 520 mosm/kg, as confirmed by the pulse labeling experiments (Fig. 5B). On the other hand, since the absolute rate of decay of ODC activity (i.e. k'PO) was very high and apparently unaffected by cycloheximide as noted above, no valid calculation of the low rate of ODC synthesis could be performed from the activity data. Nevertheless, it is quite clear that a hypertonic shock quickly reduces ODC activity levels through a dramatic repression of ODC synthesis, in conjunction with a 3-fold increase in the absolute rate of degradation of the enzyme.

The evidence presented here that hypotonic shock also results in the prolongation of the half-life of ODC in D R cells is in accordance with previous reports in other mammalian systems (2, 4). Since a significant, albeit submaximal increase in ODC half-life took place immediately following hypotonic shock (cf. Fig. 6, B and C), the mechanism responsible for decreasing the rate of ODC degradation does not have an absolute requirement for new protein synthesis and is also effective in protecting ODC molecules pre-existing before hypertonic treatment against subsequent proteolysis. The slow reversibility, together with the time-dependent increase in the extent of stabilization of ODC by hypotonic shock, could indicate a partial requirement for the additional synthesis of unknown factor(s) involved in the control of ODC degradation. The formation of a complex between ODC and its polyamine-induced antizyme has been postulated as a control point in ODC proteolysis (32). As discussed in earlier reports (9, 10), the induction of antizyme is unlikely to be a limiting step in ODC degradation in D-R cells since, as the half-life of ODC in these cells is close to that observed for the parental cell line, ODC amplification would then be expected to have occurred in concert with that of the antizyme itself. A more plausible regulatory step in the control of ODC degradation would be post-translational modifications of the enzyme such as phosphorylation (33–35). Alternatively, the capacity of the proteolytic system responsible for the degradation of ODC could approach saturation at the very high amounts of enzyme accumulated at later stages of ODC induction. Moreover, a possible stimulation of the overall rate of intracellular protein degradation resulting from a shift in substrate susceptibility to proteolysis induced by hypotonic treatment might lead to a decreased rate of ODC degradation if ODC is spared as a target by such a hypothetical effect. However, no evidence could be found for an increased rate of decay of radiolabeled, total soluble protein contents under hypoposmotic conditions (Fig. 5A and data not shown). The reason for the discrepancy observed here between the decay
rate of ODC activity and that of the immunoreactive labeled protein is not clear. The intriguing possibility that ODC activity might be affected by post-translational modifications in the absence of proteolytic attack, deserves further consideration.

The mechanism underlying the highly specific effect of hypoosmotic shock on the regulation of ODC enzyme levels is at present not understood. However, there are several noteworthy similarities between this phenomenon and the effect of polyamines on the expression of ODC. It is now well established that spermidine, spermine, and, to a much lesser degree, putrescine specifically repress the translation of ODC mRNA (10, 36-42) and accelerate the degradation of the enzyme (10, 36-40, 43) without detectable changes in the level of ODC gene transcripts (10, 36-41, 43). It is unclear how the hypoosmotically induced increase in ODC levels could be related to changes in polyamine contents. In D-R cells, as well as in several other systems (2, 4, 5), there is little, if any, variation in the intracellular contents of spermidine and spermine following hypotonic treatment, whereas putrescine levels are dramatically increased as a consequence of ODC induction. It should be pointed out, however, that relatively minor changes in polyamine content have been shown to trigger profound effects on the translation of ODC mRNA and on turnover of the enzyme in intact L1210 cells (43). As most vertebrate cell types possess some capacity for osmoregulatory volume decrease, mainly through the efflux of KCl (reviewed in Refs. 44 and 45), the lower ionic strength conditions prevailing in cells recovering from a hypotonic shock are likely to thermodynamically affect polyamine activities so as to decrease their actual free concentrations. Such a shift in intracellular polyamine activities might be evaluated by comparing the dependence of the rates of ODC mRNA translation and ODC enzyme degradation on the concentration of exogenously added polyamines under iso- and hypotonic conditions.

A most interesting parallelism can also be drawn between the induction of ODC by hypotonic shock and that caused by amino acids transported via the Na+-dependent A and N systems such as asparagine and glutamine (6, 22-25). In rat hepatocytes, supraphysiological amounts of asparagine induce regulatory volume decrease, mainly through the efflux of KCl (47-50). The rapidity of the hypotonic shock on the post-transcriptional events controlling ODC levels demonstrated in this report and that described in the case of Na+-activated amino acid transport, and possibly sugar transport (27), thus point to a mechanistic relationship, the most likely being the involvement of ionoregulatory and osmoregulatory phenomena in the reversible modulation of ODC expression.

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