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MODULATION OF MEMBRANE TRANSPORT OF ALKYLATING AGENTS AND AMINO ACIDS BY AN ANALOG OF VASOPRESSIN IN MURINE L5178Y LYMPHOBLASTS *IN VITRO**

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Abstract—The synthetic vasopressin analog 1-deamino-8-D-arginine vasopressin (dDAVP) has been shown to influence a wide range of cell-membrane-related events. Accordingly, the effect of dDAVP on membrane transport of various alkylating agents and amino acids was evaluated in L5178Y lymphoblasts *in vitro*. dDAVP stimulated melphalan uptake but conversely inhibited uptake of nitrogen mustard, choline (the natural transport substrate for the nitrogen mustard carrier), and leucine. No effect on the uptake of cyclophosphamide or glutamine was observed. Increased melphalan uptake was due to effects on both substrate influx and efflux. The effect of dDAVP on melphalan influx was particularly complex: dDAVP stimulated melphalan influx by amino acid transport system ASC but inhibited influx by system L, resulting in a net increase in unidirectional drug influx. Melphalan efflux was inhibited by dDAVP. Decreased uptake of nitrogen mustard, choline and leucine was due, at least in part, to decreased substrate influx. However, the mechanisms of inhibition were dissimilar: inhibition of substrate influx was non-competitive for choline but competitive for leucine. In conclusion, dDAVP induced diverse but apparently specific effects on membrane transport of several alkylating agents and amino acids. Since the accumulation of alkylating agents such as melphalan within tumor cells is a major determinant of cytotoxicity, dDAVP may have a role as a biological response modifier.

The synthetic vasopressin analog 1-deamino-8-D-arginine vasopressin (dDAVP‡) is known to stimulate the transport of free water across the distal nephron [1]. Other cell-membrane-related effects of dDAVP have been demonstrated recently, including the release of von Willebrand antigen, prostacyclin and plasminogen activator from vascular endothelium and factor VIII coagulant activity from hepatic sinusoids [2–6]. Correction of the bleeding time in patients with a wide range of bleeding disorders has also been demonstrated and may be due to a direct effect on endothelial cell membrane [7, 8].

The biological significance of these diverse effects remains unclear; however, the recent finding of an absent factor VIII response to dDAVP in patients with nephrogenic diabetes insipidus confirms that the dDAVP receptor is widely distributed in many tissues and may have important and diverse physiologic functions [9]. Based on these observations, we proposed that dDAVP and presumably its native counterpart may play an important physiologic role in a wide range of membrane-related events. Accordingly, the present study was undertaken to investigate the effects of dDAVP on membrane transport of the alkylating agents melphalan, HN2 and cyclophosphamide and the amino acids leucine and glutamine in murine L5178Y lymphoblasts *in vitro*.

METHODS

Drugs and chemicals. [^{14}C]Melphalan, L-*p*-(di-2-chloro[^{14}C]ethylamino)phenylalanine (specific activity 14.2 mCi/mmole) was synthesized by M. Leaffer of the Stanford Research Institute, Menlo Park, CA; the radiochemical purity was 97% as determined by thin-layer chromatography on silica gel in *n*-butyl alcohol–acetic acid–water (7:2:1) and was supplied by Dr. Robert R. Engle of the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. HN2-(2-chloroethyl-1,2-[^{14}C]) (specific activity 3.2 mCi/mmole) was obtained from the Mallinckrodt Chemical Works, St. Louis, MO. Cyclophosphamide-5-[^{14}C]-monohydrate (specific activity 2.74 mCi/mmole), choline-1,2-[^{14}C]chloride (specific activity 7.2 mCi/mmole), L-[U-^{14}C]glutamine (specific activity 2.54 mCi/mmole) and L-[4,5-^{3}H]leucine (specific activity 5 Ci/mmole) were purchased from New England Nuclear, Boston, MA. L-[^{3}H]Leucine was diluted with appropriate amounts of unlabeled L-leucine (Nutritional Biochemical Corp., Cleveland, OH) to give a specific activity of 50 mCi/mmole. The vasopressin analog dDAVP was provided by Richmond Pharmaceutical Inc., Richmond, Ontario, Canada.

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‡ Abbreviations: dDAVP, 1-deamino-8-D-arginine vasopressin; HN2, nitrogen mustard; PBS, phosphate-buffered saline; and BCH, β-2-aminobicyclo[2,2,1]heptane-2-carboxylic acid.

Transport studies. Transport studies were performed on suspension cultures of murine leukemia L5178Y lymphoblasts [10] incubated *in vitro* at cell concentrations of 2 to 4×10^6 cells/ml in Dulbecco's PBS as described previously [11–14]. The incubations were terminated by rapid chilling to 4° and centrifuging through a layer of 0.25 M sucrose in Hopkin's vaccine tubes to remove extracellular radioactivity. The washed cells were solubilized in 0.5 N NaOH, and radioactivity was determined by liquid scintillation spectrometry.

Cell size was measured in a Coulter model Z_{B1} electronic particle counter (Coulter Electronics, Hialeah, FL) calibrated with paper mulberry spores (mean cell diameter 12.5 μm) which were obtained from Coulter Diagnostics Inc. (Miami Springs, FL).

Uptake due to rapid binding to the cell membrane was determined by measuring the uptake of radiolabeled substrate at 4° at uptake times of less than 15 sec as described previously [15–18]. For all transport substrates rapid association accounted for 0.5 to 0.36 attomole/cell, which was considered negligible; accordingly the data were not corrected for this component of uptake.

Effects of dDAVP on substrate uptake. In general the effects of dDAVP on substrate uptake were determined by preincubating cells for 10 min in the presence of dDAVP and then adding the radiolabeled transport substrate at zero time. In studies of the kinetic analysis of leucine influx in the presence and absence of dDAVP, the cells were preincubated in dDAVP for 15 sec prior to the addition of [^{3}H]leucine.

Kinetic analysis of the mechanism of inhibition induced by dDAVP. A kinetic analysis of influx of choline and leucine was determined in the presence and absence of dDAVP. The kinetic parameters K_m and V_{max} were derived from linear regression equations of Lineweaver–Burk plots in which the slope represents K_m/V_{max}, the y-intercept is $1/V_{max}$, and the x-intercept is $-1/K_m$ [11–14].

The K_i for competitive inhibition was calculated from Lineweaver–Burk plots using the formula: apparent K_m (from x-intercept of curve with inhibitor present) $= K_m\,(1 + [I]/K_i)$, where K_m was obtained from the control slope in the absence of inhibitor and $[I]$ was the concentration of inhibitor, i.e. dDAVP [11, 17]. The K_i for non-competitive inhibition was determined using the formula: $1/V_{max}$ (from the y-intercept of the curve with inhibitor present) $= 1/V_{max}\,(1 + [I]/K_i)$, where $1/V_{max}$ was the y-intercept of the control curve in the absence of inhibitor and $[I]$ was the concentration of dDAVP.

Efflux studies. Efflux studies were performed on suspension cultures of L5178Y lymphoblasts incubated *in vitro* at cell concentrations of 2 to 4×10^6 cells/ml in Dulbecco's PBS, the standard transport medium. Cells were loaded with drug by incubation in 1 μM [^{14}C]melphalan for 10 min at 37° in PBS in the absence or presence of 0.1 μM dDAVP. Incubations were terminated by rapid chilling to 4° and centrifugation through a layer of 0.25 M sucrose in Hopkin's vaccine tubes to remove extracellular radioactivity. Washed cells were resuspended at 37° in a sufficient volume of fresh transport medium to minimize the problem of drug re-entry. A time–course of drug efflux was determined in the presence and absence of 0.1 μM dDAVP; aliquots were removed, chilled and centrifuged through sucrose, the cells were solubilized in 0.5 N NaOH, and radioactivity was determined by liquid scintillation spectrometry. Cell aliquots were removed prior to efflux in order to obtain the initial intracellular concentration of labeled substrate. The first order rate constant (K) for melphalan efflux was obtained by linear regression analysis of a semilogarithmic plot of a time–course of drug efflux over 5 min [19, 20]. K is equal to the negative slope of the linear regression line; the half time ($T_{1/2}$) for melphalan efflux was calculated using the equation $T_{1/2} = \ln 2/K$.

Analysis of data. All data were analyzed statistically by either a dependent or independent two-tailed t-test comparing the significance of the difference of the means. In some studies linear regression data were analyzed by a t-test comparing the significance of the difference of slopes.

RESULTS

Time course of melphalan uptake by L5178Y lymphoblasts in the presence and absence of dDAVP. A time course of melphalan uptake by L5178Y lymphoblasts demonstrated that uptake of 1 μM [^{14}C]melphalan was stimulated by the presence of 0.1 μM dDAVP (Fig. 1). Stimulation was observed over the first 2 min of the time course, when initial uptake velocity conditions were obtained, as well as from 5 to 20 min when drug uptake was in the steady state.

Dose–response effect of dDAVP on melphalan uptake by L5178Y lymphoblasts at the steady state. A study was performed to determine the effect of various concentrations of dDAVP on melphalan uptake at 10 min under steady-state conditions

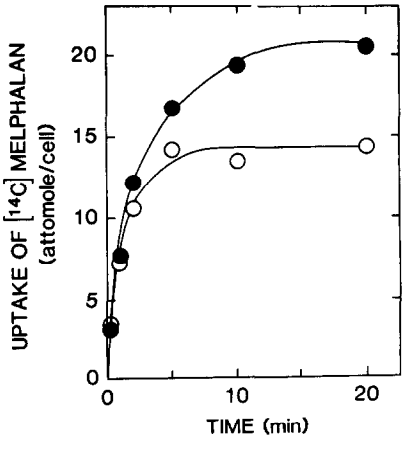

Fig. 1. Time course of the uptake of 1 μM [^{14}C]melphalan by L5178Y lymphoblasts *in vitro*, at 37°, in the presence (●) and absence (○) of 0.1 μM dDAVP. Approximately 2 to 4×10^6 cells/ml were preincubated for 10 min in PBS in the presence or absence of dDAVP before the addition of [^{14}C]melphalan. Aliquots of cell suspensions were removed at the times indicated, and radioactivity was determined by methods described in the text, and reported previously [11–14].

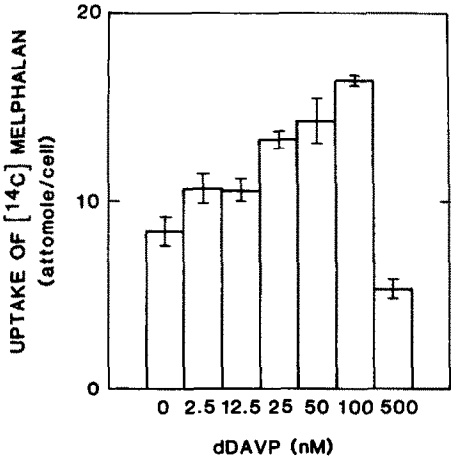

Fig. 2. Histogram demonstrating the dose–response effect of dDAVP on uptake of 1 μM [^{14}C]melphalan at 10 min in L5178Y lymphoblasts *in vitro*. Approximately 3×10^6 cells/ml were preincubated for 10 min in PBS in the presence or absence of dDAVP. Drug uptake was determined as described in the text and the legend for Fig. 1. Each bar represents the mean ± SE of four determinations; the data were analyzed by an unpaired two-tailed *t*-test.

(Fig. 2). Uptake of 1 μM [^{14}C]melphalan by L5178Y lymphoblasts was stimulated by the presence of dDAVP, and this effect followed a dose–response relationship reaching a maximum at 100 nM dDAVP. Melphalan uptake (mean ± SE) in control cells was 8.4 ± 0.8 attomoles/cell, and in the presence of 100 nM dDAVP was 16.4 ± 0.3 attomoles/cell, a 2-fold increase which was highly significant ($P < 0.001$). At higher concentrations of dDAVP, melphalan uptake decreased; this was attributed to the cytotoxic effect of 500 nM dDAVP since cell viability as measured by trypan blue dye exclusion revealed that only 66% of the cells were intact. Accordingly in all subsequent studies, unless otherwise indicated, the concentration of dDAVP used was 0.1 μM; under these conditions cell viability was greater than 95%.

Effect of dDAVP on unidirectional melphalan influx. The effect of dDAVP on melphalan influx was also studied. The rate of unidirectional melphalan influx was determined over 90 sec in the presence and absence of 0.1 μM dDAVP (Table 1). Previous studies have established that melphalan influx remains linear for approximately 2 min [13, 14, 21], so that these experimental conditions ensured that initial uptake velocity was being measured. The rate of drug influx in the intact system was increased in nine of eleven experiments by the presence of dDAVP, and this 19% increment was statistically significant.

Melphalan uptake is an active process mediated by two separate amino acid transport carriers, sodium-dependent system ASC and BCH-sensitive system L [13, 14, 22, 23]. A study was designed to determine the effect of dDAVP on each of these two transport systems (Table 1). Unidirectional melphalan influx by system ASC was measured over 90 sec in the presence and absence of dDAVP in L5178Y cells treated with 5 mM BCH, a synthetic amino acid analog that selectively blocks uptake by system L [13, 14, 24–26]. The rate of melphalan influx was increased in five of six experiments by the presence of dDAVP, and this 36% increment was statistically significant.

Melphalan influx by system L was determined in the presence and absence of dDAVP in L5178Y cells suspended in sodium-depleted medium to eliminate uptake by system ASC [27, 28]; the results are also presented in Table 1. In four of four studies, dDAVP inhibited the rate of melphalan influx by system L, and this 25% decrement of influx rate was statistically significant. Thus, dDAVP stimulated melphalan influx under conditions in which both amino acid transport systems were operative; however, only melphalan influx by system ASC was stimulated, whereas that by system L was inhibited.

Effect of dDAVP on melphalan efflux. The effect of dDAVP on melphalan efflux from L5178Y cells was investigated using methods previously described [19]. The half-time ($T_{1/2}$) for efflux of exchangeable intracellular drug increased in twelve of fourteen studies in the presence of 0.1 μM dDAVP. The $T_{1/2}$

Table 1. Effect of dDAVP on unidirectional influx rate of melphalan in L5178Y lymphoblasts *in vitro*

Transport substrate	Subst. concn (μM)	Transport medium	No. of Expt.	Substrate influx rate (attomole/cell/sec) Control*	dDAVP†	P‡
Melphalan	1	DPBS	11	0.048 ± 0.003	0.057 ± 0.005	0.05
Melphalan	1	BCH	6	0.042 ± 0.006	0.057 ± 0.005	0.036
Melphalan	1	Sodium-depleted medium	4	0.020 ± 0.003	0.015 ± 0.002	0.035

* Mean ± SE of the number of paired experiments for each set of experimental conditions shown. The influx rate was derived from a time course measuring initial velocity of drug uptake over 90 sec in the presence and absence of dDAVP. Melphalan influx in the presence of 5 mM BCH is mediated primarily by amino acid transport system ASC, whereas drug influx in sodium-depleted medium occurs mainly by system L, as described in the text.

† The concentration of dDAVP was 0.1 μM.

‡ Data were analyzed statistically by a two-tailed paired *t*-test.

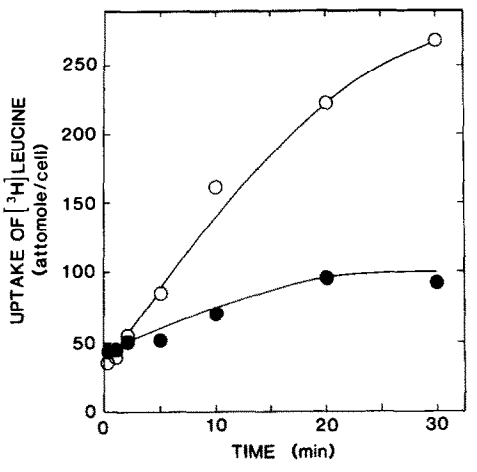

Fig. 3. Time course of the uptake of 10 μM [^{3}H]leucine by L5178Y lymphoblasts *in vitro*, at 37°, in the presence (●) and absence (○) of 0.1 μM dDAVP. Transport studies were performed using methods described in the text and the caption for Fig. 1.

(mean ± SE) for melphalan efflux from control cells was 4.7 ± 0.4 min and that from cells treated with dDAVP was 5.4 ± 0.4 min; the difference was statistically significant (P = 0.032, two-tailed paired *t*-test). Thus, the increase in melphalan uptake observed following dDAVP was due at least in part to inhibition of drug efflux.

Effect of dDAVP on amino acid uptake by L5178Y lymphoblasts. The effect of dDAVP on membrane transport of two amino acids was also examined. A time course of uptake of 10 μM [^{3}H]leucine in the presence and absence of 0.1 μM dDAVP demonstrated that leucine uptake was inhibited by dDAVP (Fig. 3). Leucine uptake at 10 min in control cells was 204.2 ± 18.5 attomoles/cell and that in the presence of dDAVP was 78.8 ± 2.9 attomoles/cell (Table 2); this 61% decrease in amino acid uptake was highly significant (P < 0.001). However, dDAVP had no effect on the uptake of glutamine (Table 2), thereby providing additional evidence for the chemical specificity of these interactions.

Effect of dDAVP on unidirectional influx of leucine. The effect of dDAVP on the rate of unidirectional influx of 10 μM [^{3}H]leucine over 90 sec was evaluated in L5178Y lymphoblasts. In three studies, the rate of unidirectional leucine influx (mean ± SE) in control cells was 0.202 ± 0.028 attomole/cell/sec, and in the presence of 0.1 μM dDAVP was 0.122 ± 0.020 attomole/cell/sec, a 40% decrement which was statistically significant (P = 0.014, two-tailed paired *t*-test).

Competitive inhibition of leucine influx by dDAVP. A kinetic analysis of the mechanism of inhibition of leucine influx by dDAVP was evaluated in L5178Y cells at 1 min over a leucine concentration range of 0.5 to 10 μM (Fig. 4). From three such experiments, the transport K_m (mean ± SE) for leucine influx in control cells was 9.0 ± 2.4 μM and that in the presence of dDAVP was 31.9 ± 7.1 μM; this 3.5-fold increase was statistically significant (P = 0.038; two-tailed unpaired *t*-test); the K_i for dDAVP as inhibitor of leucine influx was 42 ± 12 nM. The V_{max} in control cells was 29.0 ± 8.3 attomoles/cell/min, that in cells treated with dDAVP was 86.6 ± 35.9 attomoles/cell/min, and the difference was not significant. The 3.5-fold increase in K_m without a decrease in V_{max} is most consistent with the interpretation that dDAVP is primarily a competitive inhibitor of leucine influx. A confounding factor with these inhibition studies was that leucine influx in L5178Y lymphoblasts, although mediated primarily by system L, also occurs to a lesser extent by system A and a system resembling ASC [14].

Effect of dDAVP on uptake of other alkylating agents by L5178Y lymphoblasts. The effect of dDAVP on the uptake of other alkylating agents was investigated (Table 2). Uptake of 1 μM [^{14}C]HN2 by L5178Y cells was 5.5 ± 0.3 attomoles/cell and that in the presence of dDAVP was 4.2 ± 0.3 attomoles/cell; this 24% decrement in drug uptake was stat-

Table 2. Effect of dDAVP on uptake of amino acids and alkylating agents by L5178Y lymphoblasts *in vitro*

Transport substrate	Subst. concn (μM)	Uptake* (attomoles/cell) Control	dDAVP	P†
Amino acid				
Leucine	10	204.2 ± 18.5	78.8 ± 2.9	<0.001
Glutamine	1	11.3 ± 1.1	12.5 ± 1.8	NS
Alkylating agent or analog				
Melphalan	1	10.7 ± 0.5	17.3 ± 1.1	<0.001
Nitrogen mustard	1	5.5 ± 0.3	4.2 ± 0.3	<0.02
Choline	1	35.5 ± 0.9	27.4 ± 0.8	<0.001
Cyclophosphamide‡	100	37.7 ± 2.4	38.8 ± 3.7	NS

* Approximately 2 to 4 × 10^6 cells/ml were preincubated for 10 min in PBS with or without 0.1 μM dDAVP. Radiolabeled transport substrate was added at 0 time, and substrate uptake was measured at 10 min except for cyclophosphamide. Radioactivity was determined as described in the text and the legend for Fig. 1; the data represent the mean ± SE of four to six determinations.

† Data were analyzed by an unpaired two-tailed *t*-test comparing the significance of the difference of the means. NS = not significant.

‡ Cyclophosphamide uptake was measured at 30 min.

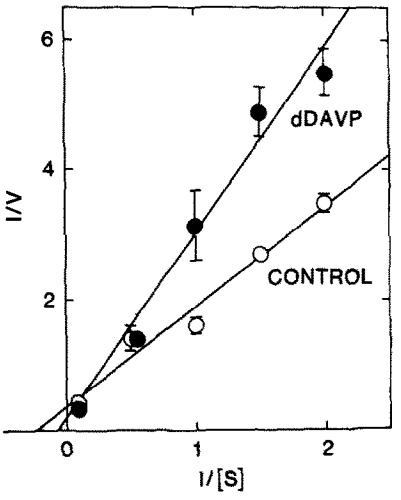

Fig. 4. Kinetic analysis of the mechanism of inhibition of leucine influx by dDAVP in L5178Y lymphoblasts. Influx of 0.5 to 10 μM [^{3}H]leucine was determined at 1 min, at 37°, in L5178Y cells after preincubation for 15 sec in PBS in the presence (●) or absence (○) of 0.1 μM dDAVP. Reciprocal uptake velocity, V, in attomoles/cell/min is plotted on the ordinate against reciprocal μM leucine concentration on the abscissa. Each point represents the mean ± SE of four determinations; on occasion the confidence intervals were too small to be illustrated. The lines were obtained by linear regression analysis. The linear regression equation for the control plot was $y = 1.55 \times 10^{17}\chi + 3.38 \times 10^{16}$ with a correlation coefficient of 0.9834 and that in the presence of dDAVP was $y = 2.88 \times 10^{17}\chi + 1.16 \times 10^{16}$ with a correlation coefficient of 0.9887.

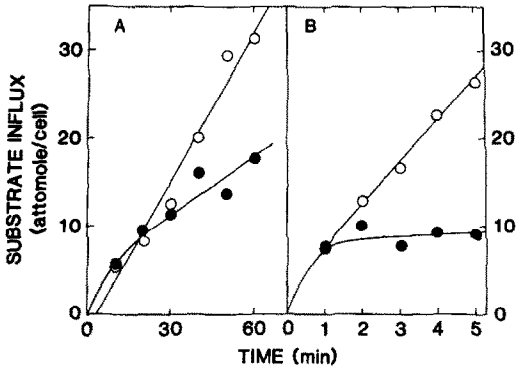

Fig. 5. Time course of unidirectional influx of 1 μM [^{14}C]HN2 (panel A) and of 1 μM [^{14}C]choline chloride (panel B) in L5178Y lymphoblasts *in vitro*, at 37°, in the presence (●) and absence (○) of 0.1 μM dDAVP. Transport studies were performed using methods described in the text and the caption for Fig. 1. Over the time period studied uptake was linear, indicating that initial uptake velocity was being measured. The regression equation for HN2 influx in the control plot was $y = 5.72 \times 10^{-19}\chi - 2.14 \times 10^{-18}$ with a correlation coefficient of 0.9825, and that in the presence of dDAVP was $y = 2.21 \times 10^{-19}\chi + 4.67 \times 10^{-18}$ with a correlation coefficient of 0.9354. The data were analyzed by a two-tailed t-test comparing the significance of the difference of the slopes. In panel B, the regression equation for choline influx in the control plot was $y = 4.72 \times 10^{-18}\chi + 3.10 \times 10^{-18}$ with a correlation coefficient of 0.9975, and that in the presence of dDAVP was $y = 1.94 \times 10^{-19}\chi + 8.28 \times 10^{-18}$ with a correlation coefficient of 0.3056. The data was analyzed by a two-tailed t-test comparing the significance of the difference of the slopes.

istically significant ($P < 0.02$). Since HN2 is actively transported on the choline carrier [11, 12], the effect of dDAVP on uptake of choline, the naturally occurring transport substrate, was studied. Uptake of 1 μM [^{14}C]choline chloride by L5178Y lymphoblasts was inhibited 23% by the presence of 0.1 μM dDAVP, and the effect was highly significant ($P < 0.001$).

The effect of dDAVP on the uptake of cyclophosphamide, an alkylating agent transported by a facilitated diffusion mechanism [18], was also evaluated. Uptake of 100 μM [^{14}C]cyclophosphamide by L5178Y cells was essentially unchanged in the presence of dDAVP (Table 2). Thus, dDAVP appeared to stimulate uptake of melphalan and to inhibit uptake of HN2; these effects were observed despite a 10-fold excess of transport substrate over modulating agent. The lack of effect on cyclophosphamide uptake suggests that these interactions are chemically specific.

Effect of dDAVP on unidirectional influx of HN2 and choline. The inhibition of uptake of HN2 and choline by dDAVP could be due to inhibition of substrate influx, stimulation of drug efflux and/or stimulation of the rate of drug inactivation and metabolism. Accordingly, the effects of dDAVP on unidirectional influx of HN2 and choline were investigated. A time course of HN2 uptake was linear for at least 60 min (Fig. 5A), suggesting that unidirectional drug influx was being measured as reported previously [11, 12]. The rate of influx of 1 μM [^{14}C]HN2 in L5178Y cells was 0.57 ± 0.05 attomole/cell/min, and that in the presence of 0.1 μM dDAVP was 0.22 ± 0.04 attomoles/cell/min; this 2.6-fold decrease in drug influx was highly significant ($P < 0.001$).

Parallel studies with 1 μM [^{14}C]choline chloride as transport substrate showed that the influx rate in control cells was 4.72 ± 0.19 attomoles/cell/min and that in the presence of 0.1 μM dDAVP was 0.19 ± 0.35 attomoles/cell/min (Fig. 5B); this 24-fold decrement was highly significant ($P < 0.001$). Thus, inhibition of uptake of HN2 and choline by dDAVP was due at least in part to inhibition of unidirectional drug influx. The influx rates of HN2 and choline initially appeared unaltered and subsequently decreased in the presence of dDAVP; this finding suggested that inhibition of unidirectional influx by dDAVP was time dependent.

Non-competitive inhibition of choline influx by dDAVP. A kinetic analysis of the mechanism of inhibition of choline influx by dDAVP was undertaken in L5178Y lymphoblasts (Fig. 6). Choline influx in L5178Y cells at 10 min over a concentration range of 1 to 10 μM was measured in the absence and presence of 0.1 μM dDAVP. A Lineweaver–Burk plot of the data revealed that inhibition was clearly non-competitive in nature. The transport K_m (mean ± SE) in control cells was 4.3 ± 1.9 μM and that in cells exposed to dDAVP was 4.1 ± 1.4 μM; the difference was not significant. The V_{max} in control cells was 11.7 ± 3.9 attomoles/cell/min and that in cells treated with dDAVP was 2.9 ± 0.7 attomoles/

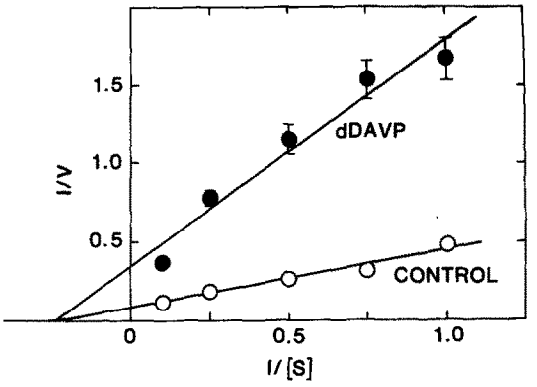

Fig. 6. Kinetic analysis of the mechanism of inhibition of choline influx by dDAVP in L5178Y lymphoblasts. Influx of 1–10 μM [^{14}C]choline chloride was determined at 10 min at 37° in L5178Y lymphoblasts after preincubation for 10 min in PBS in the presence (●) or absence (○) of 0.1 μM dDAVP. The data are plotted by the double-reciprocal method of Lineweaver–Burk with reciprocal uptake velocity, V, in moles $\times 10^{-17}$/cell/min on the ordinate against reciprocal μM substrate concentration on the abscissa. Each point represents the mean ± SE of four determinations; on occasion the confidence intervals were too small to be illustrated. The lines were obtained by linear regression analysis. The linear regression equation for the control plot was $y = 3.64 \times 10^{17}\chi + 8.53 \times 10^{16}$ with a correlation coefficient of 0.8814, and that of the plot in the presence of dDAVP was $y = 1.44 \times 10^{18}\chi + 3.50 \times 10^{17}$ with a correlation coefficient of 0.9205. The data were analyzed by a two-tailed t-test comparing the significance of the difference of the slopes and intercepts.

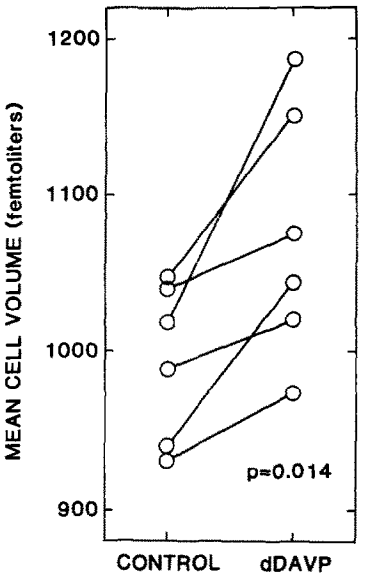

Fig. 7. Effect of dDAVP on cell volume of L5178Y lymphoblasts. The mean cell volume of L5178Y lymphoblasts was determined in the presence and absence of 0.1 μM dDAVP. Cells at a concentration of 2 to 4 $\times 10^6$ cells/ml were incubated for 20 min at 37° in PBS with or without dDAVP. Cell volume was determined by a Coulter electronic particle counter as described in the text and previously [11–14]. The data are presented as mean cell volume in femtoliters and were analyzed statistically by a two-tailed paired t-test.

cell/min; the 4-fold decrease in V_{max} was statistically significant ($P < 0.01$) and the K_i for dDAVP as inhibitor of choline influx was 32 nM.

Effect of dDAVP on cell volume of L5178Y lymphoblasts. The effect of dDAVP on the cell volume of L5178Y lymphoblasts was determined. The mean cell volume of L5178Y cells incubated in the presence and absence of 0.1 μM dDAVP was measured in a Coulter electronic particle counter (Fig. 7). In six of six determinations the cell volume increased in the presence of dDAVP; the cell volume (mean ± SE) of control cells was 994 ± 20 femtoliters, that in the presence of dDAVP was 1075 ± 33 femtoliters, and the 8% increment was statistically significant ($P = 0.014$, two-tailed paired t-test).

DISCUSSION

The vasopressin analog 1-deamino-8-D-arginine vasopressin (dDAVP) increased the uptake of the alkylating agent melphalan by L5178Y lymphoblasts but, conversely, decreased the uptake of HN2 and choline and had no effect on cyclophosphamide. Furthermore, dDAVP inhibited uptake of the amino acid leucine but had no effect on glutamine. Stimulation of melphalan uptake was due to both an increase of drug influx and inhibition of drug efflux. Inhibition of uptake of HN2, choline and leucine by dDAVP was due at least in part to inhibition of substrate influx. These findings suggest that dDAVP has diverse and yet specific effects on the transport of various alkylating agents and amino acids.

The diversity and specificity of these dDAVP-induced effects suggest that more than one effector mechanism is operative. This notion was supported by a detailed kinetic analysis of the mechanism of inhibition of choline and leucine influx by dDAVP, which revealed different effects: inhibition of choline influx was non-competitive in nature, whereas that of leucine appeared to be competitive.

The modulating effect of dDAVP on membrane transport may be due to a direct effect on the transport carrier and/or an indirect consequence of dDAVP binding to its own receptor. Since dDAVP is a polypeptide, the molecule may interact directly with amino acid carriers, such as the leucine carrier, thereby acting as a competitive inhibitor of leucine influx. The interaction of dDAVP with its receptor may result in allosteric effects on membrane proteins including changes in the number and/or mobility of transport carriers; such interactions may explain the reduction in V_{max} for choline influx, a characteristic of non-competitive inhibition.

The effect of dDAVP on melphalan influx appeared to be more complex: melphalan influx was mediated by two distinct amino acid transport carriers; a sodium-sensitive system resembling ASC and the BCH-dependent system L [13, 14, 22, 23]. dDAVP stimulated melphalan influx by system ASC but conversely inhibited influx by system L, resulting in an overall net increase in unidirectional drug influx when both transport systems were operative. A kin-

etic analysis of these effects was not done so we can only speculate on the mechanisms involved. Stimulation of melphalan influx by system ASC may represent a direct and/or indirect effect of dDAVP, resulting in increased binding affinity between melphalan and the ASC carrier and/or an increased number and/or mobility of transport carriers. Inhibition of melphalan influx by system L following dDAVP was consistent with the observed inhibition of leucine influx, since leucine transport is mediated primarily by system L in L5178Y lymphoblasts [14] as in other cells [26, 28, 29].

Although transport of the alkylating agent melphalan and the amino acid leucine by system L was inhibited by dDAVP, glutamine uptake, which is also transported by system L, was unaltered. Glutamine influx is also mediated by system ASC and system A [25, 26, 28, 29]. Thus, the absence of an effect of dDAVP on glutamine uptake does not exclude the possibility that glutamine influx by individual amino acid transport systems might be stimulated and/or inhibited, the end result, however, being no net effect on substrate uptake.

dDAVP appeared to inhibit choline influx to a much greater extent than it inhibited HN2 influx, in spite of the fact that the two compounds are substrates for the choline carrier. This effect may be partially explained by the fact that, although similar in structure, HN2 and choline are not identical molecules. In addition, the complication of alkylation reactions by HN2, an active alkylating agent, may interfere with the interpretation of these results.

Previous studies have shown that the drugs atropine, morphine and cocaine stimulate transport of choline and HN2 in L5178Y lymphoblasts [30], and in normal and leukemic human white blood cells [31]. A kinetic analysis revealed that the mechanism of stimulation was primarily due to an increase of V_{max}, that is by exposure of new carrier sites and/or stimulation of carrier mobility [30]. Maximal effects were noted when the concentration of modulating drug was 5-fold greater than that of transport substrate [30, 31]. In another study, the synthetic antiestrogen tamoxifen inhibited uptake and cytocidal activity of melphalan in human breast cancer cells *in vitro* [32]. Tamoxifen inhibits melphalan influx by system L and system ASC and also stimulates drug efflux; these effects were achieved using equimolar concentrations of tamoxifen and melphalan [32].

These comparisons emphasize the potency of the modulating effect of dDAVP on membrane transport reported in this study. Stimulation of melphalan uptake and inhibition of HN2 and choline transport were achieved at a concentration of dDAVP that was 10-fold lower than that of the transport substrate. With the amino acid leucine, inhibition of unidirectional influx was induced by dDAVP, despite a 100-fold excess of amino acid over modulating agent.

Transport regulation cannot always be explained by altered activity of transport carriers or effects on existing channels [33]. A recent morphological study using freeze-fracture electron microscopy has demonstrated that vasopressin stimulates fusion of tubular structures in the cytoplasm with apical membrane in toad urinary bladder, thereby making sites available for water movement into the cell [34]. Similar changes induced by dDAVP cannot be excluded in the present study.

The volume of L5178Y lymphoblasts was found to increase by 8% following exposure to dDAVP. These observations suggest that, in addition to effects on substrate transport, dDAVP may also affect the movement of water across the cell membrane. The changes in cell volume alone were not sufficient to explain the changes in uptake of alkylating agents or amino acids. The 8% increment in cell volume would not account for the 60% increase in melphalan uptake. Indeed the increase in cell volume would, if anything, tend to mask the reduction in uptake of HN2, choline and leucine induced by dDAVP.

At least two classes of vasopressin receptors are known [9, 35, 36]. The V_1 receptor mediates vasoconstriction of vascular smooth muscle and is responsible for the pressor effects of native arginine-vasopressin. The V_2 receptor mediates water reabsorption and is responsible for the antidiuretic effects of native vasopressin [35, 36]. dDAVP has a specificity for the V_2 rather than the V_1 receptor [36, 37]; accordingly, the effects observed in this study may be V_2 mediated.

Recently melphalan has been used in conjunction with autologous bone marrow transplantation in the treatment of patients with neuroblastoma and other solid tumours [38–40]. Since the accumulation of alkylating agents within tumor cells is a major determinant of cytotoxicity, the observed increase in melphalan uptake by L5178Y lymphoblasts induced by dDAVP may have potential clinical applicability; dDAVP may have a role as a biological response modifier.

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