REEVALUATION OF RESERPINE-INDUCED SUPPRESSION
OF CONTACT SENSITIVITY

Evidence that Reserpine Interferes with T Lymphocyte Function
Independently of an Effect on Mast Cells

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Reserpine profoundly inhibits delayed hypersensitivity responses in a variety
of experimental systems (1–9). Schnyder and Storck (1) initially proposed that
reserpine suppressed contact sensitivity (CS) through an autonomic or central
nervous system effect which interfered with the afferent limb of the immune
response. This view was consistent with the best-known pharmacological property
of the drug, depletion of stores of catecholamines and serotonin (5-hydroxytrypt-
tamine) (5-HT) in the brain and other sites, but was not supported by subsequent
work of other investigators. For example, Polak and Turk reported that reserpine
did not alter the afferent limb of CS (3). They found instead that the drug
suppressed the efferent limb of the response, an effect they thought might be
due to peripheral vasoconstriction and diminished vascular permeability. Ger-
shon et al. (4) and Askenase et al. (5) confirmed that reserpine interfered with
cellular immunity by blocking expression of the efferent limb of the response,
but suggested yet another mechanism to account for this effect. They proposed
that elicitation of delayed hypersensitivity (DH) reactions required the T cell-
directed release of 5-HT from mast cells at the site of antigenic challenge, and
that reserpine interrupted this sequence by depleting mast cell granules of 5-
HT.

This hypothesis accommodated a variety of experimental findings (4–9), but
is difficult to reconcile with several more recent observations. At least four
different groups have reported that DH responses can be expressed in the virtual
absence of tissue mast cells. Three of these groups found that W/W° (10–13) or
S1/S1d (12, 13) mice, whose tissues contain <1% the normal numbers of mast
cells (14, 15), and which cannot express detectable passive cutaneous anaphylaxis (10, 16), nevertheless developed DH responses whose intensity equalled or exceeded those in littermate control mice with normal numbers of mast cells. A fourth group also confirmed the occurrence of DH responses in W/W v and S1/S1 d mice, although in these experiments DH was associated with less swelling in mast cell-deficient mice than in littermate controls (16). Furthermore, reserpine blocked the swelling (12, 16) and the leukocyte infiltration (12) associated with delayed hypersensitivity responses in mast cell-deficient mice, suggesting that the drug’s ability to abrogate DH may be independent of its effect on mast cells.

These observations prompted us to reevaluate the mechanism by which reserpine interferes with the expression of cellular immunity. We now report evidence for a previously unrecognized action of reserpine that can explain its ability to block expression of cellular immunity either in the presence or in the absence of tissue mast cells: the inhibition of effector T cell function.

Materials and Methods

Mice. Female BALB/c, C57BL/6J, and mast cell-deficient mice (WB/Re]-W/+ × C57BL/6J-W/+)+F1 (W/W v, +/+) were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were used at 6-10 wk of age.

Reagents. 2,4-Dinitro-l-fluorobenzene (DNFB) was obtained from Eastman Kodak Co. (Rochester, NY). 4-Ethoxymethylene-2-phenyl oxazalone (Ox) was purchased from BDH Chemicals Ltd. (Poole, England). Reserpine USP solution (serpasil 2.5 mg/ml), was purchased from CIBA Pharmaceutical Co. (Summit, NJ).

Elicitation and Quantitation of Contact Sensitivity Response. Mice were sensitized to DNFB by applying 25 μl of 0.5% DNFB in acetone/olive oil (4:1 by volume) to the shaved abdomen on days 0 and 1. Mice were sensitized to Ox by applying 50 μl of 3.0% Ox in acetone/olive oil (4:1 by volume) to the shaved abdomen on days 0 and 1. Unless otherwise specified, contact sensitivity reactions were elicited by challenging mice on both sides of the left ear with 20 μl of either 0.2% DNFB or 0.5% Ox (10 μl to each side of the ear, 5 d after sensitization) in a vehicle of 4:1 acetone/olive oil. The right (control) ear received acetone/olive oil alone.

The effect of systemic administration of reserpine on the expression of contact sensitivity was demonstrated by administering the drug (5 mg/kg, i.p.) 18 h before ear challenge (4, 12). Control animals were injected with 0.9% NaCl alone.

The contact responses were measured by an ear swelling assay. In most experiments, the reactions were also quantitated by determining ear weight ratios and by a radiometric assay of leukocyte migration. (a) Ear swelling: the mice were lightly anesthetized with ether and the thickness of the ears was measured immediately before challenge and 24 h after challenge with a Mitutoyo engineer’s micrometer (13, 17). The increment (Δ) of swelling (24 h value - baseline value) was expressed in units of 10⁻⁴ inches. (b) Ear weight ratio: mice were killed by cervical dislocation 24 h after challenge; the ears were cut off at the hairline and trimmed carefully so that each ear was a mirror image of the other. The contact response of each mouse was expressed as the ratio of the weight of the left (challenged) ear and right (control) ear (12, 13). A few transfer experiments required challenging both ears with antigen. In these experiments, the results were recorded as the actual weights of the ears in each experimental group. (c) Radiometric assay of leukocyte emigration: immediately after challenge, mice were injected intraperitoneally with 0.1 ml of 10⁻⁷ M 5-fluoro-2-deoxyuridine (FUDR) and, after an additional 30 min, with 2 μG of 125I-5-iodo-2-deoxyuridine (125I-IUDR) sp act, 2,200 G/mmol; (New England Nuclear, Boston, MA [18, 19]). The ears were amputated, trimmed, and weighed as described above and then were washed to deplete the tissue of free 125I-labeled species as previously described (12, 13). The radioactivity remaining in the washed ears was counted in a gamma counter (No. 1185; Tracor Analytic, Inc., Elk Grove Village, IL).
response of each mouse was expressed as the ratio of counts per minute of challenged to that of control ears according to the formula: (cpm left ear − background)/(cpm right ear − background). For all three assays, the results are expressed as mean ± SE.

Antigen-induced Proliferation of Immune Lymph Node Cells (I-LNC) In Vitro. Mice were sensitized with Ox as described above. On day 5, the axillary, brachial, mandibular, popliteal, and inguinal lymph nodes of the sensitized mice were removed aseptically, teased over a metal screen in RPMI 1640 medium (Gibco, Grand Island, NY) at 20°C, and then washed twice with RPMI medium. The cells [I-LNC(Ox)] were then resuspended (1.0 × 10⁶ cells/ml) in RPMI-FCS (RPMI medium supplemented with glutamine [0.3 mg/ml], 2-mercaptoethanol [2.5 × 10⁻³ M], gentamycin [10 μg/ml], and 10% fetal calf serum [M. A. Bioproducts, Walkersville, MD]). The cells were then distributed into 96-well flat-bottomed tissue culture plates (Tissue Culture Cluster; Costar, Cambridge, MA) at 2.0 × 10⁵ cells (in 200 μl RPMI-FCS) per well.

[3H]Thymidine [3H]TdR incorporation was used as a measure of lymphocyte proliferation. To augment lymphocyte proliferation, some wells received antigen (Ox₃₅-human serum albumin [Ox₃₅-HSA], prepared according to the method of Yoshimura and Cinader [20]) given in 200 μl of RPMI-FCS to achieve the final concentrations indicated in Results. Some wells also received reserpine (in 5 μl of RPMI-FCS) to achieve the final concentrations indicated in Results. The cells were then placed in a humidified atmosphere of 5% CO₂/95% air at 37°C for 3 d. [3H]TdR (6.7 Ci/mmol; New England Nuclear) was then added (1 μCi/well) for an additional 24 h. The cells were then harvested onto glass fiber filters (No. 23-994; M. A. Bioproducts), the filters placed in Aquasol (New England Nuclear), and the disintegrations per minute of incorporated [3H]TdR was measured in a Tacor Mark IV β counter.

Transfer of Contact Sensitivity. DNFB-immune lymph node cells I-LNC(DNFB) were obtained from donor mice sensitized on days 0 and 1 with a total of 55 μl of 0.5% DNFB in 4:1 acetone/olive oil (25 μl applied to the shaved abdomen, and 5 μl to each of the feet and ears). I-LNC(Ox) were obtained from donor mice sensitized with a total of 80 μl 3% Ox in 4:1 acetone/olive oil (50 μl to the abdomen, and 5 μl to each of the feet and ears). On day 4 (for DNFB) or day 5 (for Ox) the draining lymph nodes were removed and single-cell suspensions were prepared and washed in RPMI medium. Recipients were given 5 × 10⁷ I-LNC intravenously. Mice were ear challenged within 1 h of transfer unless otherwise specified. For cotransfer experiments I-LNC from two different donor groups (5 × 10⁷ of each type of I-LNC) were mixed and injected intravenously into the same mouse.

In Vitro Treatment of I-LNC with Reserpine to Block Transfer of Contact Sensitivity. I-LNC prepared as above were diluted to a concentration of 5 × 10⁶ cells/ml in RPMI medium, pH 7.2, supplemented with 5% FCS. Reserpine was added in various final concentrations (as indicated in Results) and the reaction was allowed to proceed for 60 min at 37°C in a humidified atmosphere of 5% CO₂, 95% air. Control I-LNC were incubated in the same manner but without the addition of reserpine. Viability of cells at the end of each incubation (with or without reserpine) was assessed by trypan blue exclusion and was found to be >90% in all experiments. The reserpine-treated or untreated cells were then concentrated by centrifugation, resuspended in fresh RPMI medium, counted, and then immediately transferred intravenously or intradermally into naive mice. Generally, the ears were challenged for CS within 1 h of passive transfer of the I-LNC.

Quantitation of Reserpine-induced Inhibition of Contact Sensitivity. For measurements of ear swelling, percent inhibition of the contact response was calculated as: [(swelling in mice given control I-LNC) − (swelling in mice given reserpine-treated I-LNC)]/(swelling in mice given control I-LNC) × 100. For ear weight or [125I]IUDR ratios, percent inhibition of the contact response was calculated as: [(ratio of mice given control I-LNC − 1.0) − (ratio of mice given reserpine-treated I-LNC − 1.0)]/(ratio of mice given control I-LNC − 1.0) × 100.

[125I]IUDR Incorporation by Spleen, Lymph Node, or Bone Marrow Cells In Vivo. [125I]IUDR is incorporated into all rapidly dividing cells, including those in the spleen, lymph nodes, and bone marrow (21). In assays of DH, ratios of [125I]IUDR cpm in challenged and control
ears primarily reflect differences in $^{125}$I-labeled leukocytic infiltration (12, 18). Furthermore, histological analyses indicate that most of the leukocytes which infiltrate CS reactions are bone marrow derived (i.e., granulocytes and monocytes [12, 18]). If, for whatever reason, $^{125}$I-IUDR incorporation by bone marrow cells is affected by the reserpine treatment, then the $^{125}$I-IUDR ratios obtained in these mice might be altered artifically. In some experiments, we therefore removed one femur from each mouse at the time of sacrifice, trimmed the femurs of soft tissue, washed them three times in ethanol (13, 18), and then determined their radioactivity in a gamma counter as described above. In these experiments we also determined incorporation of $^{125}$I-IUDR by spleen or lymph node cells. The spleens and axillary lymph nodes were removed at the time of sacrifice, minced, washed three times in ethanol, and their radioactivity measured in a gamma counter.

**Measurement of Histamine and Serotonin.** Duplicate aliquots of I-LNC (5.0 × 10^6 cells per determination) were assayed for histamine content fluorometrically with an Autoanalyzer II (Technicon Instruments Corp., Tarrytown, NY) equipped to detect histamine in the 0.5–10 ng/ml range (22). Duplicate aliquots of I-LNC (5.0 × 10^6 cells per determination) were assayed for serotonin (5-HT) content by the highly sensitive and specific enzymatic-isotopic method of Hammel et al. (23), in which 5-HT is converted to [H]-melatonin in a two-step reaction (25). The relationship of dpm to 5-HT is linear in the 0.5–10 ng range (23). Internal standards of histamine or 5-HT (both from Sigma Chemical Co., St. Louis, MO) were used in each assay (22, 23).

**Statistical Analysis.** The results of ear swelling assays, measurements of actual ear weights, and determinations of $^{125}$I-IUDR incorporation into spleen, lymph node, or bone marrow cells, were compared for statistical significance ($P < 0.05$) using Student's $t$ test (two-tailed). The Mann-Whitney U test (two-tailed) was used to evaluate the significance ($P < 0.05$) of differences in measures of CS based on ratios between the left (challenged) and right (control) ears.

**Results**

**Systemic Treatment with Reserpine Blocks Expression of Contact Sensitivity and Incorporation of $^{125}$I-IUDR into Lymph Node Cells.** As described by Gershon et al. (4), reserpine given intraperitoneally (5 mg/kg) 18 h before challenge for contact sensitivity virtually abolished the animal's ability to develop a CS response, whether judged by measures of tissue swelling or $^{125}$I-labeled leukocyte emigration (Table I). At this dose, the drug also produced other remarkable

| Group | Reserpine treatment | Contact sensitivity responses | Incorporation of $^{125}$I-IUDR |
|-------|---------------------|-------------------------------|---------------------------------|
|       |                     | Ear weight ratio (L/R)        | Bone marrow cells               |
|       |                     | Ear $^{125}$I-IUDR ratio (L/R)| Spleen cells                    |
|       |                     |                               | Lymph node cells                |
| A     | −                   | 2.22 ± 0.06                   | 2.752 ± 201                     |
| B     | +                   | 1.04 ± 0.04                   | 2.455 ± 585                     |

$P < 0.01$ vs. A  $P < 0.008$ vs. A  $P < 0.008$ vs. A

BALB/c mice were sensitized by epicutaneous application of oxazolone on days 0 and 1. Group B received reserpine (5 mg/kg, i.p.) 18 h before challenge of the left ear (L) with oxazolone on day 5. The right ear (R) received vehicle alone and served as a control. Mice in group A were injected with 0.9% NaCl instead of reserpine. FUDR and $^{125}$I-IUDR (2 μCi/mouse, i.p.) were administered within 30 min of ear challenge with antigen. 24 h contact sensitivity reactions were quantitated by determining the weight ratios and $^{125}$I-IUDR ratios of challenged and control ears. The $^{125}$I-IUDR incorporation of femoral bone marrow cells, spleen cells, and lymph node cells was also determined. The results are shown as the mean ± SE, and were tested for statistical significance by the two-tailed Mann-Whitney U test or two-tailed Student's $t$ test as described in the text. NS, not significant ($P > 0.05$).
Reserpine inhibits baseline and antigen-augmented levels of $[^3]$H$]Tdr$ incorporation by I-LNC(Ox) in vitro. I-LNC(Ox) were recovered from C57BL/6J mice that had been sensitized to Ox 5 d previously. The I-LNC(Ox) were incubated with or without specific antigen (Ox$_{ss}$-HSA) for 3 d, at which time $[^3]$H$]Tdr$ was added for the final day of culture. Some populations also were treated with reserpine, which was given at the time of antigenic challenge and every 24 h thereafter. The addition of specific antigen significantly augmented $[^3]$H$]Tdr$ incorporation by I-LNC not exposed to reserpine ($P < 0.005, <0.001, \text{or } <0.001$ when results with 10, 100, or 200 $\mu$g Ox$_{ss}$-HSA/ml were compared with results with no added antigen by two-tailed Student's $t$ test). Reserpine markedly diminished $[^3]$H$]Tdr$ incorporation at all concentrations tested ($P < 0.001$ when results with any dose of reserpine were compared to results in the absence of the drug at any concentration of antigen (0, 10, 100, or 200 $\mu$g/ml) by two-tailed Student's $t$ test).

Reserpine Inhibits Lymphocyte Incorporation of $[^3]$H$]Tdr$ In Vitro. Because of experiments such as that shown in Table I, and because of reports suggesting that reserpine may have antiproliferative effects in other systems (24, 25), we tested the effect of the drug on $[^3]$H$]Tdr$ incorporation by I-LNC in vitro. We first administered the drug to I-LNC 30 min before the introduction of specific antigen (Ox), and at daily intervals thereafter (total of four administrations). The result is shown in Fig. 1. Reserpine nearly eliminated $[^3]$H$]Tdr$ incorporation by I-LNC incubated without Ox-HSA, and also markedly reduced the augmented levels of isotope incorporation exhibited by cells stimulated with

**Figure 1.** Reserpine inhibits baseline and antigen-augmented levels of $[^3]$H$]Tdr$ incorporation by I-LNC(Ox) in vitro. I-LNC(Ox) were recovered from C57BL/6J mice that had been sensitized to Ox 5 d previously. The I-LNC(Ox) were incubated with or without specific antigen (Ox$_{ss}$-HSA) for 3 d, at which time $[^3]$H$]Tdr$ was added for the final day of culture. Some populations also were treated with reserpine, which was given at the time of antigenic challenge and every 24 h thereafter. The addition of specific antigen significantly augmented $[^3]$H$]Tdr$ incorporation by I-LNC not exposed to reserpine ($P < 0.005, <0.001, \text{or } <0.001$ when results with 10, 100, or 200 $\mu$g Ox$_{ss}$-HSA/ml were compared with results with no added antigen by two-tailed Student's $t$ test). Reserpine markedly diminished $[^3]$H$]Tdr$ incorporation at all concentrations tested ($P < 0.001$ when results with any dose of reserpine were compared to results in the absence of the drug at any concentration of antigen (0, 10, 100, or 200 $\mu$g/ml) by two-tailed Student's $t$ test).
additional specific antigen in vitro. At the concentrations tested, reserpine had no effect on lymphocyte viability (viability of cells on day 4 was always >90% by trypan blue exclusion). Additional similar experiments (data not shown) demonstrated that reserpine largely blocked (>90% inhibition) baseline or antigen-stimulated [³H]TdR incorporation by I-LNC derived from BALB/c or (C57BL/6J × A/J)F₁ mice at doses as low as 0.25 μg/ml (0.4 μM), when added to the cultures on a daily basis, and at doses as low as 2.5 μg/ml (4 μM) when administered only once at the beginning of culture, 30 min before the addition of antigen.

Gershon et al. (4) suggested that reserpine abrogated the expression of T cell–dependent responses indirectly, by depleting stores of 5-HT in mast cells. Examination of our BALB/c I-LNC preparations after toluidine blue or Wright's staining showed that they did contain a few mast cells (<0.1%). They also contained detectable amounts of 5-HT (84 ng per 1.0 × 10⁶ cells). We therefore decided to rule out the possibility that reserpine's ability to inhibit I-LNC proliferation in vitro was mediated indirectly, by an effect of the drug on this minor population of mast cells. We prepared I-LNC(Ox) from W/Wv mice whose tissues contained <1% the number of mast cells of their littermate controls (14), and from similarly sensitized littermate control (+/+ ) mice with normal numbers of mast cells. W/Wv I-LNC preparations contained no mast cells by toluidine blue or Giemsa staining. In addition, these preparations contained no detectable histamine (<0.5 ng per 1.0 × 10⁶ cells) or 5-HT (<0.5 ng per 1.0 × 10⁶ cells). By contrast, rare mast cells (<0.1%) were present in +/+ I-LNC preparations, and these populations contained detectable amounts of both histamine (6.6 ng per 1.0 × 10⁶ cells) and 5-HT (57 ng per 1.0 × 10⁶ cells). As shown in Fig. 2, a single administration of reserpine (4 μM) at the beginning of culture markedly inhibited both baseline and antigen-augmented [³H]TdR incorporation by I-LNC derived from W/Wv or +/+ mice. This result indicates that reserpine's effect on lymphocyte [³H]TdR incorporation does not require the intervention of mast cells.

Reserpine Treatment of I-LNC In Vitro Blocks Their Ability to Transfer CS. The T cells that proliferate in vitro when restimulated with specific antigen represent a different population than the T cells capable of passively transferring contact sensitivity (26). Furthermore, Gershon et al. (4) found that lymphocytes from mice sensitized to sheep red blood cells and treated with reserpine 8 h before sacrifice were as effective in passively transferring DH reactivity as were lymphocytes from sensitized mice not treated with the drug. This result was interpreted as evidence that the significant effect of reserpine was "on cells of the host, not on the immunocompetent cells of the donor" (4). However, standard transfer studies use sensitized lymphocytes that are washed before injection into naive recipients, and our studies of I-LNC [³H]TdR incorporation in vitro indicated that the effects of reserpine on lymphocyte proliferation were partially reversible (data not shown). We therefore determined whether reserpine could interfere with the function of effector cells that transfer CS, using an approach which avoided washing of the treated cells.

In the first series of experiments, immune lymph node cells from mice sensitized to oxazolone I-LNC(Ox) were incubated for 1 h at 37°C with different
FIGURE 2. Reserpine inhibits baseline and antigen-augmented levels of \[^{3}H\]Tdr incorporation by I-LNC(Ox) independently of an effect on mast cells. The experiment was similar to that shown in Fig. 1, except that I-LNC(Ox) were derived from mast cell–deficient W/W\(^{v}\) mice or from littermate control (+/+) mice with normal numbers of mast cells. Reserpine (4 \(\mu\)M) was administrated once at the beginning of culture, and 24-h \[^{3}H\]Tdr incorporation was determined 3 d later. Specific antigen (100 \(\mu\)g/ml) augmented \[^{3}H\]Tdr incorporation by W/W\(^{v}\) or +/+ I-LNC (\(P < 0.001\) compared to corresponding results without antigen, by two-tailed Student’s t test). Reserpine virtually eliminated \[^{3}H\]Tdr incorporation by W/W\(^{v}\) or +/+ I-LNC at all concentrations of antigen (0, 10, or 100 \(\mu\)g/ml) tested (\(P < 0.001\) by two-tailed Student’s t test).

concentrations of reserpine. The cells were then centrifuged, resuspended in fresh RPMI medium, and immediately injected intravenously into each of five syngeneic recipients (5.0 \(\times\) 10\(^{7}\) cells per mouse). Control cells included I-LNC(Ox) not treated with reserpine, and lymph node cells recovered from mice not sensitized to Ox (naive lymph node cells, N-LNC). These populations were incubated, resuspended, and injected in the same manner as reserpine-treated I-LNC(Ox). Recipient animals were challenged within 1 h of cell transfer by applying antigen to the left ear, and the contact responses were measured 24 h later.

At a concentration of 25 \(\mu\)g per 1.0 \(\times\) 10\(^{6}\) cells/ml (40 \(\mu\)M), reserpine treatment produced 64% inhibition of the contact response according to micrometer measurements of ear swelling, 100% inhibition according to ear weight ratios, and 96% inhibition according to \(^{125}\)I-IUDR ratios (experiment 1, Table II). It should be pointed out that the contact responses elicited with reserpine-treated I-LNC(Ox) were even smaller than those elicited in mice which received naive-LNC, as judged by the results of micrometer measurements or ear weight ratios. When the data are viewed in this fashion (i.e., after subtraction of the nonspecific responses elicited in mice that received N-LNC), it is evident that reserpine treatment of I-LNC in vitro completely abolished the transfer of CS.

In experiment 2 (Table II), we tested the effect of treating I-LNC(Ox) with 20 \(\mu\)M reserpine (12.5 \(\mu\)g reserpine per 5.0 \(\times\) 10\(^{6}\) cells/ml). Since 5.0 \(\times\) 10\(^{7}\) LNC were transferred to each mouse, animals in the reserpine treatment group received I-LNC that had been incubated with a total of 125 \(\mu\)g of reserpine.
Reserpine Treatment of I-LNC Abrogates Their Ability to Transfer Contact Sensitivity

Table II

| Exp | Donor cells | Reserpine treatment | Left ear swelling (mm) | Ear weight ratio (L/R) | Ear $^3$H-IUDR ratio (L/R) |
|-----|-------------|---------------------|------------------------|------------------------|---------------------------|
| 1   | A. I-LNC(Ox) | —                   | 61 ± 6                 | 1.38 ± 0.04            | 3.09 ± 0.28               |
|     | B. I-LNC(Ox) | 25 µg/ml            | 15 ± 6                 | <0.001 vs. A          | 1.00 ± 0.02               | <0.004 vs. A               |
|     | C. Naive-LNC | —                   | 18 ± 2                 | <0.001 vs. A          | 1.08 ± 0.02               | <0.004 vs. A, NS vs. B    |
| 2   | A. I-LNC(Ox) | —                   | 55 ± 6                 | 1.56 ± 0.07            | 3.43 ± 0.32               |
|     | B. I-LNC(Ox) | 12.5 µg/ml          | 18 ± 2                 | <0.001 vs. A          | 1.09 ± 0.02               | <0.01 vs. A, NS vs. B     |
|     | C. Naive-LNC | —                   | 14 ± 2                 | <0.001 vs. A          | 1.08 ± 0.02               | <0.01 vs. A, NS vs. B     |

I-LNC(Ox) were recovered from BALB/c mice sensitized to Ox 5 d previously. N-LNC were from BALB/c mice not sensitized to Ox. The I-LNC(Ox) were incubated with or without reserpine for 1 h at 37°C before adoptive transfer into naive BALB/c recipients (see text for further details). Mice were challenged on the left ear with Ox, and on the right (control) ear with vehicle alone, within 1 h of cell transfer. Mice then received FUDR and $^3$H-IUDR intraperitoneally, and the magnitude of the CS reactions was determined 24 h later. NS, not significant ($P > 0.05$).

Reserpine Blocks Transfer of CS Independently of an Effect on Mast Cells. Gershon et al. (4) proposed that the abrogation of CS by reserpine reflected the drug's ability to deplete mast cell stores of 5-HT. One could therefore argue that reserpine-treated I-LNC simply transferred sufficient drug to impair the function of tissue mast cells at the site of antigen challenge in the recipient mice. To test this possibility, we performed an experiment in which I-LNC(Ox) derived from mast cell–deficient W/W° mice were transferred into naive W/W° mice. W/W° mouse I-LNC(Ox) contained no mast cells by toluidine blue or Wright's staining, and contained undetectable amounts of histamine (<0.5 ng per 1.0 x 10⁶ cells) or 5-HT (<0.5 ng per 1.0 x 10⁶ cells). Nevertheless, the W/W° I-LNC were fully competent to transfer CS to W/W° recipients, and treatment of the I-LNC with reserpine (20 μM) for 1 h completely inhibited the ability of these cells to transfer CS reactivity (Fig. 3). This experiment indicated that W/W° mice represent suitable donors and recipients for the transfer of CS. In addition, reserpine blocked the transfer of CS under conditions where both the I-LNC preparations and the naive recipient mice were essentially devoid of mast cells. This result...
FIGURE 3. Reserpine blocks transfer of CS in W/W' mice. The experiment was similar to those shown in Table II, except that mast cell-deficient W/W' mice were used both as a source of I-LNC(Ox) and as recipients for the transfer of CS. Recipient mice received either I-LNC(Ox) (group A), I-LNC(Ox) exposed to reserpine (20 μM) for 1 h in vitro (group B), or, as a control, I-LNC derived from W/W' mice sensitized to the unrelated antigen DNFB (group C). (*) Reserpine, 12.5 μg per 5 × 10^6 cells/ml for 1 h. Mice were challenged with antigen (left ears) or vehicle (right ears) within 1 h of cell transfer, and the intensity of the CS reaction was determined 24 h later (see text for details). W/W' mice that received I-LNC(Ox) developed CS reactions (group A). By contrast, W/W' mice that received I-LNC(Ox) treated with reserpine (group B) exhibited reactivity to Ox which was statistically indistinguishable from that seen in mice which received I-LNC from animals sensitized to an unrelated antigen (group C).

strongly suggests that reserpine blocks the transfer of CS independently of an effect on mast cells.

We also measured the 125I-IUDR incorporation into the femoral bone marrow, spleen, and axillary lymph nodes of the same recipient W/W' mice depicted in Fig. 3. In contrast to systemic treatment with reserpine (5 mg/kg), which profoundly diminished LNC incorporation of 125I-IUDR (Table I), passive transfer of reserpine-treated I-LNC had no effect on the low levels of 125I-IUDR incorporation exhibited by the LNC of the naive recipient mice (the values for groups A, B, and C were 51 ± 9, 48 ± 10, and 58 ± 14 cpm). These groups of mice also exhibited little or no differences in the levels of 125I-IUDR incorporation by bone marrow cells (the values for groups A, B, and C were 1,696 ± 201, 1,600 ± 241, and 1,704 ± 36) or spleen cells (the values for groups A, B, and C were 2,693 ± 159, 2,749 ± 276, and 3,504 ± 191).

Reserpine-induced Suppression of CS Is Not Due to Systemic Effects of Drug Transferred with the Treated I-LNC. Reserpine blocked the transfer of CS by I-LNC (a) when used at concentrations that produced only mild sedative effects in recipients of the treated cells, and (b) when mast cell-deficient W/W' mice were used both as the source of I-LNC and as the naive recipients in a transfer experiment. However, one could still explain these results by postulating that I-LNC incubated with reserpine simply transferred sufficient drug to block CS by a systemic action on a host cell population other than mast cells.

To test this possibility, we performed an experiment in which 5 × 10^7 I-LNC from mice sensitized to DNFB [I-LNC(DNFB)] were cotransferred with 5 × 10^7 reserpine-treated or untreated [I-LNC(Ox)] (Fig. 4). The left ears of recipient mice were then challenged with Ox, while the right ears were challenged with DNFB. Mice that received both reserpine-treated I-LNC(Ox) (12.5 μg reserpine per 5.0 × 10^6 cells/ml) and untreated I-LNC(DNFB) (group B) exhibited significant suppression of the response to Ox (73% suppression by ear swelling mea-
RESERPINE-INDUCED INHIBITION OF CONTACT SENSITIVITY

Figure 4. The inhibition of transfer of CS by reserpine is specific for the treated cells. Naive BALB/c mice received a mixture of $5 \times 10^7$ I-LNC(Ox) and $5 \times 10^7$ I-LNC (DNFB) (group A), a mixture of $5 \times 10^7$ I-LNC(Ox) that had been treated with reserpine (20 µM) for 1 h in vitro and $5 \times 10^7$ I-LNC(DNFB) (group B), or $1 \times 10^8$ lymph node cells obtained from normal BALB/c mice (N-LNC, group C). (*) Reserpine, 12.5 µg per $5 \times 10^6$ cell/ml, for 1 h. All mice were challenged within 1 h of cell transfer by applying Ox to the left ear and DNFB to the right ear. The CS reactions were measured 24 h later by determining the thickness (ear swelling [$\times 10^{-4}$ inches]) and weight of the challenged left and right ears. Mice that received I-LNC(Ox) treated with reserpine (group B) exhibited no more reactivity to Ox than did control mice which received naive-LNC (group C), whether judged by the swelling or the actual weight of left ears challenged with Ox. But reactivity to DNFB was the same in mice that received I-LNC(Ox) treated with reserpine (group B) as in mice which received I-LNC(Ox) not treated with the drug (group A). This result indicates that the reserpine transferred with the treated I-LNC(Ox) had no detectable effect on the function of I-LNC(DNFB) transferred into the same mice.

Reserpine-induced Suppression of CS Is Not Due to Local Effects of Drug Transferred with the Treated I-LNC. The results in Fig. 4 do not prove that the critical effect of reserpine in blocking CS is an effect on T cells. For example, in mice that received reserpine-treated I-LNC(Ox), the local concentrations of the drug may have been higher in the Ox-challenged ear (if the reserpine-treated cells migrated to that site) than in the contralateral ear challenged with DNFB. According to this scenario, host cells necessary for expression of CS may have been affected by the relatively high concentrations of reserpine present in ears challenged with Ox. We therefore performed an experiment to determine whether the important effect of reserpine in blocking transfer of CS was its effect on the treated, sensitized T cells, or its effects on other cells at the site of the CS reaction.

We first evaluated whether reserpine's ability to block expression of CS could be demonstrated when the treated I-LNC were injected directly into the ear. We found that pretreatment of I-LNC(Ox) with 20 µM reserpine for 1 h had no effect on the viability of the cells, as determined by measurement of their ability...
Reserpine Treatment Blocks Transfer of Contact Sensitivity Even When I-LNC Are Directly Injected into the Site of Antigen Challenge

| Donor cells | Challenge | Contact sensitivity responses |
|-------------|-----------|------------------------------|
|             |           | Ear swelling (A X 10^-4 inches) | P | Ear weight (mg) | P |
| A. I-LNC(Ox) | Ox | 25 ± 2 | 40.3 ± 0.6 | <0.001 vs. A, NS vs. C or D | <0.001 vs. A, NS vs. C or D |
| B. I-LNC(Ox)-Res | Ox | 11 ± 1 | 35.8 ± 0.5 | <0.001 vs. A, NS vs. C or D | <0.001 vs. A, NS vs. C or D |
| C. I-LNC(Ox) | Vehicle | 7 ± 1 | 34.7 ± 0.8 | <0.001 vs. A, NS vs. B or D | <0.001 vs. A, NS vs. B or D |
| D. I-LNC(Ox)-Res | Vehicle | 9 ± 1 | 35.5 ± 0.5 | <0.001 vs. A, NS vs. B or C | <0.001 vs. A, NS vs. B or C |

I-LNC(Ox) were recovered from BALB/c mice sensitized to Ox 5 d previously, and were incubated with reserpine [I-LNC(Ox)-Res] or without reserpine [I-LNC(Ox)] (20 μM; 12.5 μg per 5.0 x 10^6 cells/ml) for 1 h at 37°C. The cells were then centrifuged, resuspended in RPMI medium, and injected intradermally (2.0 x 10^6 cells in 20 μl/site) into the dorsal surface of the ears of naive BALB/c recipients. The ears were challenged with Ox (0.2%; 20 μl to the dorsal surface only) or with vehicle, within 1 h of cell injection. The magnitude of the CS reactions was determined 24 h later. NS, not significant (P > 0.05).

Local Intradermal Injection of Reserpine-treated I-LNC(Ox) Does Not Interfere with the Development of CS to DNFB at the Same Site

| Group | Donor cells | Challenge | Contact sensitivity responses (ear swelling, Δ X 10^-4 inches) |
|-------|-------------|-----------|-------------------------------------------------------------|
|       | Left ear | Right ear | Left ear | Right ear | Left ear | Right ear |
| A.    | I-LNC(Ox) | I-LNC(Ox) | Ox | DNFB | 28 ± 2 (P < 0.001) | 28 ± 2 (P < 0.001) |
| B.    | I-LNC(Ox)-Res | I-LNC(Ox)-Res | Ox | DNFB | 11 ± 2 (NS vs. C) | 11 ± 2 (NS vs. C) |
| C.    | I-LNC(Ox) | I-LNC(Ox)-Res | Vehicle | Vehicle | 10 ± 1 | 10 ± 1 |

The experiment was performed as described in the legend of Table IV, except that all recipient mice were sensitized to DNFB by epicutaneous application of 0.5% DNFB (25 μl per application) on days 0 and 1. Untreated I-LNC(Ox), or cells treated with 20 μM reserpine for 1 h [I-LNC(Ox)-Res], were injected intradermally into the ears on day 4. The ears were challenged with 20 μl of either Ox (0.2%), DNFB (0.2%), or vehicle within 1 h of cell injection. The magnitude of the CS reactions was determined 24 h later. NS, not significant (P > 0.05).

to exclude trypan blue, but virtually eliminated their ability to orchestrate a CS response in vivo (Table III). This result indicates that reserpine can block expression of CS even under circumstances which eliminate the need for migration of sensitized T cells to the site of antigen challenge.

We next performed an experiment similar to that shown in Table III, except that the recipient mice were first actively sensitized to DNFB. We then injected reserpine-treated or untreated I-LNC(Ox) directly into the ears of these mice, and challenged some ears with Ox and some with DNFB. The results of this experiment are shown in Table III. Reserpine-treated I-LNC(Ox) exhibited normal viability by trypan blue exclusion and by cell counts, but were unable to transfer a significant CS response after intradermal injection (compare values...
Reserpine does not induce suppressor activity in the treated I-LNC population. In this cotransfer experiment, mice received either $5.0 \times 10^7$ I-LNC(Ox) (group A), $5.0 \times 10^7$ I-LNC(Ox) treated with reserpine (20 $\mu$M) for 1 h in vitro (group B), or a mixture of $5.0 \times 10^7$ reserpine-treated I-LNC(Ox) and $5.0 \times 10^7$ untreated I-LNC(Ox) (group C). (*) Reserpine, 12.5 $\mu$g per $5 \times 10^6$ cells/ml, for 1 h. All mice were challenged on the left ear with antigen (Ox) and on the right (control) ear with vehicle, within 1 h of cell transfer. The CS responses were measured 24 h later. Mice that received only I-LNC(Ox) treated with reserpine (group B) developed reactions that were much weaker than those in mice that received only untreated I-LNC(Ox) (group A). By contrast, CS reactions in mice that received a mixture of the treated and untreated cells (group C) were statistically indistinguishable from the reactions in animals which received only untreated cells (group A).

For Ox-challenged left ears in groups A–C, Table IV). By contrast, the presence of reserpine-treated I-LNC(Ox) had no significant effect on the development of CS reactions to DNFB (compare values for right ears challenged with DNFB). This experiment, like that in Table III, showed that reserpine-treated I-LNC were unable to orchestrate a CS response even when the treated cells were directly injected into the site of antigen challenge.

The experiment also showed that non-T cell populations required for expression of CS (e.g., host accessory cells) were sufficiently active at sites injected with reserpine-treated I-LNC(Ox) to support the development of CS reactions to DNFB. It is likely that such cells also would have been adequate to collaborate with sensitized T cells in the development of a CS reaction to Ox. But reactions did not develop at sites injected with reserpine-treated I-LNC(Ox) and challenged with Ox. This finding suggests that no matter what effects reserpine might have on the function of non-T cells in the I-LNC preparations, the consequence of drug treatment which is responsible for reserpine's ability to block CS is an effect on T cells.

Reserpine-induced Inhibition of Transfer of CS Is Not Due to Activation of Suppressor Cells. Epicutaneous sensitization of mice induces at least two distinct T cell subpopulations (27, 28): effector T cells of the CS reaction (TDH) and auxiliary suppressor T cells (Ts$_{aux}$). The latter population is required for suppression of the efferent limb of CS by suppressor T cells (Ts$_{off}$ [28]). Because the I-LNC populations we transferred would be expected to contain both TDH and Ts$_{aux}$, we considered the possibility that inhibition of transfer of immunity by reserpine reflected an ability of the drug to induce expression of suppressor activity by Ts$_{aux}$.

We evaluated this possibility with a cotransfer experiment. I-LNC were prepared as described above and part of the population was treated with reserpine (12.5 $\mu$g per $5 \times 10^6$ cells/ml), whereas the rest of the cells were incubated for a similar period (1 h) without the drug. As shown in Fig. 5, naive mice that received
$5 \times 10^7$ untreated I-LNC(Ox) (group A) developed CS responses when challenged with the antigen. By contrast, mice that received the same number of I-LNC(Ox) treated with reserpine (group B, Fig. 5) developed little or no CS reactions. The last group of mice (group C) received a mixture (1:1) of reserpine-treated and untreated I-LNC(Ox) (a total of $1.0 \times 10^8$ cells per recipient). These mice developed 24-h CS responses that were statistically indistinguishable from those in mice which received only untreated I-LNC(Ox). This finding argues against the participation of reserpine-activated suppressor cells (with or without specificity for antigen) in this system. Moreover, it also reinforces the notion that reserpine-induced suppression of the transfer of CS is specific for the treated I-LNC, and does not reflect a systemic effect of the small amount of reserpine transferred to the recipients with the treated cells.

We also investigated the role of suppressor cell activation in reserpine-induced inhibition of CS using a different approach. Cyclophosphamide selectively depletes suppressor cell precursors (29) and specifically eliminates the suppressive effect induced by Taux (28). We found that pretreatment of mice with cyclophosphamide (200 mg/kg [28]) 2 d before active sensitization had no detectable effect on the abrogation of the CS responses induced by reserpine (5 mg/kg, i.p.) given either 6 or 18 h before challenge (data not shown). Together with the results of the cotransfer experiment discussed above, these findings suggest that activation of suppressor T cells is unlikely to be the mechanism by which reserpine inhibits the expression of CS.

**Washing of Reserpine-treated I-LNC Restores Their Ability to Transfer CS.** We next determined whether the reserpine's effect on the transfer of CS with I-LNC was reversible. For these studies, we used a modification of an approach described by Moorhead (30). I-LNC were treated with reserpine in vitro for 1 h as described above (12.5 $\mu$g/5 $\times 10^6$ cells/ml). Some of the treated cells were then concentrated by centrifugation, resuspended in fresh medium, and immediately injected intravenously into syngeneic recipients. The remaining reserpine-treated I-LNC were resuspended in RPMI medium containing 5% FCS, but no reserpine, for a 1-h incubation at 37°C. These cells were then washed twice in RPMI medium with 5% FCS (150 g, 10 min, room temperature), resuspended in RPMI medium and injected intravenously into recipients. Control I-LNC not treated with reserpine were handled the same way (half the cells transferred after a 1-h incubation at 37°C, half the cells given an additional 1 h incubation in RPMI/5% FCS and then two washes). All recipients were ear challenged within 1 h of cell transfer and the CS responses were measured 24 h later.

The results are shown in Fig. 6. As expected, mice that received I-LNC immediately after the cells had been treated with reserpine for 1 h developed little or no CS response to antigenic challenge; they exhibited 79% suppression of CS by ear swelling measurements, and 92% suppression of CS by $^{125}$I-IUDR ratios compared with I-LNC transferred after a similar incubation without the drug (Fig. 6, group A vs. B). By contrast, mice that received reserpine-treated I-LNC that were incubated for an additional hour without the drug and then washed (group D) exhibited CS responses that were statistically indistinguishable from those observed in mice which received washed (group C) control cells. This
Reserpine-Induced Inhibition of Contact Sensitivity

**TABLE V**

Reserpine Treatment Blocks Transfer of Contact Sensitivity Even When Antigen Challenge Is Delayed Until 4 h After Injection of I-LNC

| Cells         | Time of Ox challenge (h) | Left ear swelling ($\times 10^4$ inches) | Ear weight ratio (L/R) | Ear $^{125}$I-UdR ratio (L/R) |
|---------------|-------------------------|----------------------------------------|------------------------|-------------------------------|
|               |                         | $P$                                     | $P$                    | $P$                           |
| A. I-LNC(Ox)  | 1                       | 54 ± 2                                  | 1.59 ± 0.05            | 3.54 ± 0.19                  |
| B. I-LNC(Ox)-Res | 1                       | <0.001 vs A                            | 1.12 ± 0.01            | 1.29 ± 0.08                  |
| C. I-LNC(Ox)  | 4                       | NS (<0.1)                               | 1.48 ± 0.05            | 4.15 ± 0.54                  |
| D. I-LNC(Ox)-Res | 4                       | <0.001 vs C                            | 1.22 ± 0.04            | NS vs A                       |

I-LNC(Ox) were recovered from BALB/c mice sensitized to Ox 5 d previously, and were incubated with [I-LNC(Ox)-Res] or without [I-LNC(Ox)] reserpine (20 μM) for 1 h at 37°C. The cells then were transfused, without prior washing, into naive BALB/c recipients. Mice were challenged on the left ear with Ox, and on the right (control) ear with vehicle alone, 1 or 4 h after cell transfer. Mice received FUDR and $^{125}$I-UdR intraperitoneally within 30 min of Ox challenge, and the magnitude of the CS reactions was determined 24 h later. NS, not significant ($P > 0.05$).

Experiment indicates that reserpine-induced abrogation of the ability of I-LNC to transfer CS is reversible.

We also tested whether reversibility of the drug effect might be demonstrated simply by delaying antigenic challenge of mice that received reserpine-treated and unwashed I-LNC. The results of this experiment, shown in Table V, indicate that reserpine treatment of I-LNC largely blocked the ability of these cells to transfer CS even when antigenic challenge was delayed until 4 h after cell transfer.
Discussion

We found that reserpine inhibited at least two of the three T cell populations activated during sensitization for contact sensitivity. Reserpine profoundly inhibited the T cells (T_{prolif} [26, 27]) which proliferate in vitro when restimulated with the antigen used for epicutaneous sensitization. Reserpine also markedly interfered with the transfer of contact sensitivity responsiveness to naive syngeneic recipients by immune lymph node cells (I-LNC).

The latter finding suggested that the drug inhibited the activity of the T cell population capable of conferring reactivity for delayed hypersensitivity (T_{DH} [27]). But reserpine can exert antiinflammatory effects independently of its actions on T cells. It blocks expression of passive cutaneous anaphylaxis, probably by its ability to deplete mast cells of 5-HT (4). It also inhibits the tissue swelling and leukocyte emigration associated with immunologically nonspecific reactions to contactants (3, 12), effects observed even in mice lacking mast cell- or platelet-derived 5-HT (12). In the present experiments, we were concerned that some of the suppression of CS observed in mice that received reserpine-treated I-LNC may have been due to T cell–independent antiinflammatory actions of the reserpine transferred with the treated cells. We therefore attempted to prove that the important effects of the drug in our experiments were due to its actions on the adoptively transferred I-LNC, rather than to systemic or local effects on mediators or cellular circuits in the recipient animals.

First, we considered the hypothesis that reserpine blocks the expression of CS and other T cell–mediated immune responses by depleting mast cells of the vasoactive amine serotonin 5-HT (4, 5, 9, 16). The results of our experiments indicate that this is a most unlikely explanation of reserpine’s effect in CS. The I-LNC preparations used in our studies contained <0.1% mast cells according to staining with toluidine blue or Wright’s stain and they contained only small amounts of 5-HT or histamine. Moreover, I-LNC from mast cell–deficient W/W^v mice, cell preparations that contained no detectable mature mast cells, 5-HT, or histamine, responded to treatment with reserpine in vitro in a manner indistinguishable from that of I-LNC derived from their normal (+/+) littermate controls. Thus, reserpine markedly inhibited both baseline and antigen-augmented incorporation of [3H]TdR by I-LNC derived from either W/W^v mast cell–deficient or littermate control (+/+) mice. Furthermore, I-LNC derived from W/W^v mice and exposed to 20 μM reserpine for 1 h in vitro failed to transfer CS reactivity to naive W/W^v mice. By contrast, aliquots of the identical preparation of I-LNC that had not been treated with reserpine were fully competent to transfer CS to native W/W^v mice. We feel that these findings, together with the observation that systemic administration of reserpine inhibits DH responses in W/W^v mast cell–deficient mice (12, 16), indicate that reserpine can block expression of CS independently of an effect on mast cells.

We then showed that the inhibition of CS responsiveness was specific for the treated I-LNC. First, animals that received both reserpine-treated I-LNC from mice sensitized to Ox [I-LNC(Ox)] and I-LNC from mice sensitized with DNFB [I-LNC(DNFB)] expressed little or no CS to Ox challenge, but responded normally to challenge with DNFB on the opposite ear. Second, we showed that the local intradermal injection of reserpine-treated I-LNC(Ox) did not detectably
interfere with the development of CS to DNFB at the same site. By contrast, ears injected with reserpine-treated I-LNC(Ox) did not develop detectable CS reactions after challenge with Ox, a result that also indicates that reserpine’s ability to block expression of CS cannot be explained simply by an effect of the drug on T cell migration to the site of antigen challenge. Finally, we tested the effect of transferring reserpine-treated I-LNC(Ox) together with I-LNC(Ox) not treated with the drug. Mice that received a 1:1 mixture of the reserpine-treated and the untreated cells responded to antigen challenge in a manner indistinguishable from that of mice that received untreated I-LNC(Ox) alone. By contrast, mice that received only reserpine-treated cells developed little or no CS response. This confirms our I-LNC(Ox) and I-LNC(DNFB) cotransfer experiment in demonstrating no systemic effect of the reserpine transferred with the treated cells. It also suggests that the reserpine treatment of I-LNC did not induce or augment suppressor cell activity in the treated population.

Two lines of evidence suggest that the drug did not have a cytolytic effect on the treated cells. First, reserpine had no effect on the viability of I-LNC, as judged by cell counts and by examining the ability of these cells to exclude trypan blue. Second, the effect of the drug on Tdth function in vivo was fully reversible. Washing of reserpine-treated I-LNC was required to restore their ability to transfer CS; simply transferring the treated I-LNC into naive mice, a maneuver that resulted in a dilution of the small amount of drug injected with the treated cells, did not have the same effect. It may be of interest that a similar distinction between the consequences of washing cells in vitro and injecting them in vivo has been reported for the inhibitory effect of specific hapten on transfer of CS by I-LNC (30). But further work will be required to determine whether there is any relationship between the mechanisms responsible for the reversibility of reserpine-induced, as opposed to hapten-induced, inhibition of I-LNC function.

We do not know the precise mechanism by which reserpine interferes with I-LNC activity. Although a direct effect on T cells appears likely, studies with cloned effector T cells will be required to rule out an indirect effect mediated by other elements in the I-LNC preparations. It will also be of interest to define exactly which manifestations of T cell function are affected by reserpine, and which of these are critical for the expression of CS in vivo. The transfer of CS and other DH responses requires that the sensitized T cells perform a complex functional program, which includes migration to sites of antigen challenge, recognition of specific antigen presented in an immunologically appropriate context, and elaboration of mediators, not yet fully characterized, which amplify and focus the inflammatory response. Many of these activities require the collaboration of host cells (e.g., vascular endothelial cells, antigen-presenting/processing cells, and leukocytes without immunological specificity). It is possible that reserpine has more than one effect on T cell function, and may influence some critical non-T cell functions as well. Put differently, nothing we have done rules out the possibility that reserpine can interfere with cell-mediated immunity through redundant effects on multiple cells involved in the reaction. Nevertheless, our cotransfer experiments showed that reserpine’s ability to block CS cannot easily be explained by systemic or local effects of the drug on non-T cell populations of the host. By contrast, our work strongly suggests that reserpine
has effects on sensitized T cells which are sufficient to account for the drug's ability to block cell-mediated immune responses.

Summary

It has been suggested that reserpine blocks expression of delayed hypersensitivity (DH) by depleting tissue mast cells of serotonin (5-HT), thereby preventing a T cell-dependent release of mast cell 5-HT necessary to localize and to amplify the DH response. However, reserpine blocks expression of DH in mast cell-deficient mice. We therefore decided to reevaluate the mechanism by which reserpine abrogates expression of cellular immunity, and investigated whether the drug might interfere with T cell activity in vitro or in vivo. At concentrations as low as 4 μM, reserpine profoundly suppressed baseline or antigen-augmented levels of [3H]thymidine incorporation by immune lymph node cells obtained from mice sensitized to the contactant oxazolone [I-LNC(Ox)]. This effect was observed both with I-LNC derived from normal mice and with I-LNC derived from congenitally mast cell-deficient W/Wv mice, cell preparations that lacked detectable mast cells, histamine, and 5-HT.

Furthermore, treatment of I-LNC with reserpine (20 μM) for 1 h in vitro virtually abolished the ability of these cells to transfer CS to naive mice. This was not a cytolytic effect, as the viability of the I-LNC treated with reserpine was not affected, and washing of the reserpine-treated I-LNC before transfer fully restored their ability to orchestrate a CS response. The action of the drug was not mediated by an effect on mast cells, since the experiment could be performed using mast cell-deficient W/Wv mice as both donors and recipients of I-LNC. In addition, the effect was specific for the treated cells: mice that received reserpine-treated I-LNC(Ox) intravenously together with untreated I-LNC(DNFB) did not develop CS to Ox but responded normally to DNFB; and local intradermal injection of reserpine-treated I-LNC(Ox), which failed to transfer reactivity to Ox, did not interfere with the development of CS to DNFB at the same site. Finally, cotransfer experiments indicated that the effect of reserpine on the transfer of CS was not due to activation of suppressor cells. Our findings strongly suggest that whatever effects reserpine might have on immunologically nonspecific host cells, the drug's effects on sensitized T cells are sufficient to explain its ability to block cell-mediated immune responses in vivo.

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