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Neurotransmitter Suppression of the In Vitro Generation of a Cytotoxic T-lymphocyte Response Against the Syngeneic MOPC-315 Plasmacytoma[1]

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by

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Footnotes

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Abstract

The effects of norepinephrine and related molecules on the generation of antitumor cytotoxic activity were determined. Cytotoxic activity was generated by coculture of normal spleen lymphocytes from BALB/c mice with syngeneic MOPC-315 plasmacytoma cells and was assayed by the ^{51}Cr release assay. At concentrations 100 μM, the catecholamines norepinephrine, epinephrine, isoproterenol and dopamine inhibited the development of cytotoxic activity by 50-90%. However, at concentrations of 0.1 to 1 μM, norepinephrine but not the other catecholamines enhanced the generation of cytotoxicity. Generation of anti-MOPC-315 cytotoxicity was also enhanced by the non-catecholamines serotonin and carbachol (50-100 μM). Serotonin and carbachol at concentrations of 0.1 to 100 μM did not inhibit the generation of antitumor cytotoxicity. The generation of antitumor cytotoxicity was inhibited by the second messenger dibutyryl cAMP (500 μM). Norepinephrine inhibited the generation of cytotoxic activity only when added during the first two days of a 5-day coculture period, whereas dibutyryl cAMP inhibited the generation of cytotoxic activity when added up to 4 days after the culture was initiated. The inhibitory effects of norepinephrine on the generation of cytotoxicity were not blocked by the β-adrenergic antagonist propranolol. These studies suggest that a stress-induced elevation of catecholamines may suppress the generation of anti-tumor cytotoxic activity. Such a mechanism may help to explain the effects of stress on tumor progression and eradication.

Introduction

Lymphoid organs are innervated by sympathetic neurons. The presence of synapse-like junctions between neurons and lymphocytes (1, 2) indicates that lymphocytes are exposed to neurotransmitters in situ. Norepinephrine, the classical sympathetic neurotransmitter, is present in high concentration in the spleen (2). The presence of adrenergic receptors on lymphocytes suggests that norepinephrine can participate in neuro-immunomodulation. Many reports suggest that physiological or psychological stress can affect immune function. For example, Fawzy et al. (3) have shown that psychiatric intervention reduced psychologic distress, enhanced longer-term coping and enhanced the numbers and cytotoxic activity of natural killer cells in patients with malignant melanoma. Other reports demonstrate that surgical, environmental, physiological, and psychosocial stress impair antitumor immunity (4-6). Such stress may diminish antitumor immunity through a mechanism(s) involving activation of the sympathetic nervous system and the release of norepinephrine and/or other sympathetic neurotransmitters (7).

Immunoregulation by norepinephrine in vivo is suggested by the observation that sympathetic denervation of the spleen enhances antibody production (8-10), albeit through undefined mechanisms. Consistent with these in vivo studies catecholamine neurotransmitters have also been shown to inhibit the proliferation of B-lymphocytes and T-lymphocytes in vitro (11-13). In our

previous studies, it was observed that there are different mechanisms of catecholamine inhibition in B cells and T cells (11). The catecholamine inhibition of T and B-cell DNA synthesis was not blocked by adrenergic or dopaminergic receptor antagonists.[3]

The current study was undertaken to determine whether stress-related neurotransmitters can inhibit the generation of cytotoxic T-lymphocyte (CTL) responses against a syngeneic tumor. The focus of our attention on the generation of CTL activity against tumor cells arises from the growing appreciation of the importance of the CTL lytic mechanism in tumor eradication (14-19). As a prototype of a tumor model in which the CTL plays an important role in in vivo tumor eradication, we selected the MOPC-315 tumor model. Since the exact conditions for the in vitro generation of a CTL response are well established in the MOPC-315 tumor model, this model provides an ideal system to study the effects of catecholamines on the generation of antitumor cytotoxicity (20). In this study, the MOPC-315 tumor system was used to study the effects of catecholamines, non-catecholamine neurotransmitters and associated molecules on the generation of a CTL response in vitro.

Materials and Methods

Animals. Animals used for maintenance of tumor cells and as a source of spleen cells for the in vitro generation of cytolytic activity were 7 to 10 week old female BALB/c mice purchased from Charles River Breeding Laboratories, Wilmington, MA.

Chemicals. All neurotransmitters and related compounds used in these studies were purchased from Sigma, St. Louis, MO.

Tumor Cells. MOPC-315 plasmacytomas were maintained in vivo as subcutaneous tumors as previously described (20). Mice were inoculated subcutaneously with $1x10^6$ viable tumor cells. Single cell suspensions were prepared by mechanical disruption of the tumor between glass slides (20). The viability, as determined by trypan blue dye exclusion, always exceeded 95%.

Spleen Cell Suspensions. Suspensions of spleen cells from normal mice were prepared by mincing spleens and gently pressing the cells through a sterile Nytex nylon mesh (Tetko, Inc., Emsford, NY) with a sterile stainless steel lab spoon (American Scientific Products, McGaw Park, IL). The single cell suspensions were washed and resuspended in culture medium for use in the in vitro stimulation. In any individual experiment, pooled spleen cells from at least 5 mice were used.

In Vitro Stimulation for the Generation of Cytotoxic T-lymphocytes. MOPC-315 tumor cells were treated with mitomycin C (50 μg/ml) for 30 minutes and washed three times. Mitomycin C-treated tumor cells ($1.33x10^6$) were admixed with spleen cells

($40x10^6$) from normal BALB/c mice and cultured in 20 ml of medium consisting of RPMI 1640 supplemented with 1% nonessential amino acids (#320-1140, Gibco, Grand Island, NY), 2 mM glutamine, 50 μM 2-mercaptoethanol (Sigma, St. Louis, MO), 50 Units/ml penicillin, 50 μg/ml streptomycin (Sigma) and 5% heat-inactivated fetal bovine serum (Gibco). Agonists were added at the time of culture initiation unless otherwise stated. The cultures were incubated at 37°C in 5%CO_2-air for 5 days unless otherwise stated since the generation of the CTL response peaks between days 4-6 (20, 21).

Cytolytic Assay. The cytolytic activity of the cultured spleen cells was determined by the ^{51}Cr release assay as previously described (20). Briefly, the cultured spleen cells were washed three times and incubated for 3.5 hours with 5×10^4 ^{51}Cr-labelled target MOPC-315 cells in 12 x 75 mm plastic tubes at effector to target cell (E/T) ratios of 200:1, 100:1, 50:1, and 25:1. At the end of the incubation period, the cells were pelleted, and radioactivity in the supernatants (Sup) and pellets (Pel) was counted in a gamma scintillation counter. The percentage of ^{51}Cr release for each sample was calculated as follows:

$$\% \text{ of } ^{51}Cr \text{ release} = \frac{\text{cpm in Sup}}{\text{cpm in Sup + cpm in Pel}} \times 100$$

The percentage of specific ^{51}Cr release was calculated by the following formula:

$$\% \text{ of specific } ^{51}Cr \text{ release} = \frac{T - C}{M - C} \times 100$$

where T is the percentage of release with test lymphocytes, C is the mean value calculated for three replicates of the percentage of

spontaneous release (which ranged between 16 and 30%), and M is the mean value calculated for three replicates of the percentage of maximal release (obtained after addition of 2% NP-40), which ranged between 82 and 97%. Each experiment was performed a minimum of two times. The level of antitumor cytotoxicity is presented as the mean ± SEM from triplicate samples. The SEM did not exceed 6%. Although the level of antitumor cytotoxicity generated by lymphoid cells varied from one experiment to another (22-24), the pattern of the results remained consistent and reproducible. Probability values of $p<0.05$ as determined by a completely random Anova followed by Dunnett's t Test were considered statistically significant.

Results

Catecholamine Agonist Inhibition of the Generation of Cytotoxic Activity Against MOPC-315 Tumor Cells. The effects of catecholamines on the generation of a cytotoxic T-cell response against a syngeneic tumor were examined. Norepinephrine, epinephrine, or isoproterenol was added to BALB/c spleen cells at the time of initiation of a 5-day culture with mitomycin-C-treated MOPC-315 tumor cells. As a control, spleen cells were cultured with MOPC-315 cells in the presence of HCL, the solvent for the neurotransmitters. At the concentrations used, HCl had no effect on the generation of cytotoxicity. At high concentrations (100 μM), all of the catecholamines greatly inhibited (60-72% inhibition) the generation of anti-MOPC-315 cytotoxicity (Fig.1). This inhibition was evident when the lytic activity was assayed at effector to target cell (E/T) ratios of 200:1, 100:1 and 50:1. The cytotoxicity exerted by the norepinephrine-treated cells at an E/T ratio of 200:1 was lower than that exerted by control cells at an E/T ratio of 50:1. The dramatically reduced anti-tumor cytotoxicity following stimulation in the presence of the catecholamines was not a consequence of drug toxicity since the drugs did not affect cell viability as determined by trypan blue exclusion and by fluorescein diacetate fluorescence (measured after 2 days of culture) nor did they reduce the number of lymphocytes harvested on day 5. Figure 1 also shows that at concentrations of 0.1-1 μM the generation of anti-MOPC-315 cytotoxicity was augmented

(30-50%) by norepinephrine but not by epinephrine or isoproterenol.

The Effects of Dopamine on the Generation of Antitumor Cytotoxic Activity. Since dopamine can act through its own dopamine receptors (25) as well as adrenergic receptors, the effects of dopamine on the 5 day generation of antitumor cytotoxicity was also investigated. Figure 2 reveals that dopamine (50 and 100 μM) inhibited the generation of anti-MOPC-315 cytotoxicity by 30-85%. The decrease in generation of antitumor cytotoxicity with dopamine (50-100 μM) was not due to toxicity since dopamine did not affect the cell yield on day 5 of the culture. In contrast to norepinephrine, lower concentrations of dopamine (0.1-1 μM) did not affect the generation of anti-MOPC-315 cytotoxicity.

The Effects of Non-catecholamine Neurotransmitters on the Generation of Antitumor Cytotoxic Activity. Experiments were performed to investigate whether non-catecholamine neurotransmitters could affect the generation of anti-MOPC-315 cytotoxicity. Serotonin or carbachol (a cholinergic agonist) was added at the initiation of a 5 day culture for the in vitro generation of anti-tumor activity. Figure 2 shows that, in contrast to catecholamines, serotonin and carbachol at concentrations between 0.1 μM to 100 μM did not inhibit the generation of cytotoxic activity although some enhancement was noted.

Cyclic-AMP Parallels the Catecholamine Inhibition of the Generation of Antitumor Cytotoxicity. Our previous studies suggest

that cAMP is the second messenger for catecholamines in T cells (11). To investigate the role of cAMP as a second messenger in the inhibition of lymphocyte activation by catecholamines, we examined whether dibutyryl cAMP, a membrane penetrable analog of cAMP, could act in ways similar to catecholamines. Figure 3 reveals that, at concentrations previously shown to inhibit other lymphocyte functions (26), dibutyryl cAMP did indeed inhibit the generation of cytotoxic activity against MOPC-315 tumor cells. Since inhibition by norepinephrine may be mediated via cAMP it was important to determine if both compounds have the same kinetics of inhibition. Norepinephrine or dibutyryl cAMP was added to spleen cells immediately prior to addition of the mitomycin-C-treated MOPC-315 tumor cells. Cytotoxic activity against MOPC-315 cells was assessed on days 3, 4, and 5 of the culture. Figure 4 shows that over 90% inhibition by norepinephrine and dibutyryl cAMP was seen by day 3. Further, cAMP and norepinephrine had parallel effects on the generation of antitumor cytotoxicity.

Effect of Delayed Addition of Norepinephrine and Dibutyryl cAMP on the Inhibition of the Generation of Antitumor Cytotoxicity. Experiments were performed to find out if norepinephrine and cAMP inhibit the same "stage" of the generation of antitumor cytotoxicity. Accordingly, we determined whether norepinephrine and dibutyryl cAMP affects only early or later events in the generation of antitumor cytotoxicity. Norepinephrine or dibutyryl cAMP was added to spleen cells and mitomycin-C-treated MOPC-315 cells on days 0, 1, 2, 3, or 4 and cytotoxic activity was assessed

simultaneously for all treatment groups on 5 day of the culture. Figure 5 illustrates that maximal inhibition of the generation of cytotoxic activity occurred upon addition of norepinephrine at the beginning of the in vitro stimulation, inhibition was noted when norepinephrine was added on day 1 or 2, and no inhibition could be seen when it was added on day 3 or 4. In contrast, generation of cytotoxic activity was drastically inhibited when dibutyryl cAMP was added up to 4 days after culture initiation. Thus, while norepinephrine was inhibitory only when added at early stages of the generation, dibutyryl cAMP was inhibitory when added at early or late stages in the generation of cytotoxic activity against MOPC-315 tumor cells.

Analysis of the Participation of the β-Adrenergic Receptor in Catecholamine Inhibition of the Generation of Antitumor Cytotoxicity. Since adenylyl cyclase which catalyzes the production of cAMP is coupled to β-adrenergic receptors, experiments were designed to determine whether catecholamines inhibited the generation of cytotoxic activity via binding to β-adrenergic receptors found on lymphocytes (27-29). For this purpose, the ability of propranolol, a β-adrenergic antagonist, to block the inhibitory effect of norepinephrine on generation of antitumor cytotoxicity was studied. Spleen cells were incubated with propranolol for 30 minutes prior to the addition of norepinephrine and mitomycin-C-treated MOPC-315 cells. After 5 days in culture, cytotoxic activity was measured. Figure 6 shows that propranolol (0.1 μM to 10 μM) did not block the

norepinephrine-induced (100 μM) inhibition of the generation of anti-MOPC-315 cytotoxicity by spleen cells. This lack of antagonistic effect by propranolol did not result from an inability of the mixed population of spleen cells to respond to propranolol since propranolol blocked isoproterenol stimulation of adenylyl cyclase activity by aliquots obtained from the same preparation of spleen cells (data not shown), a result observed previously.[3]

Discussion

It has been demonstrated that the anti-MOPC cytotoxicity is due to MOPC-315 specific $CD8^+$ CTL cells generated in vitro (30-33). The studies in this report demonstrate that the catecholamines isoproterenol, norepinephrine, epinephrine and dopamine but not the non-catecholamines serotonin or carbachol can suppress the in vitro generation of a CTL response against the syngeneic MOPC-315 plasmacytoma. These molecules were inhibitory at a concentration of 100 μM, a nontoxic concentration not unexpected for localized concentrations of norepinephrine in the spleens of stressed mice. The concentration of norepinephrine in the 6 nm synapse-like junctions between neurons and T-lymphocytes is probably much higher than the 1 μM interstitial norepinephrine concentration in mouse spleen (2). Moreover, under stress, sympathetic stimulation increases norepinephrine release in the spleen (34). Furthermore, although the participation of the known β-adrenergic receptors in the inhibition of anti-MOPC-315 cytotoxicity is unclear, the K_D for norepinephrine dissociation from β-adrenergic receptors present on lymphocytes (100-400 μM) is similar to the "effector" catecholamine concentrations reported here (27, 28).

The catecholamine inhibition of the generation of anti-MOPC-315 cytotoxic activity was mimicked by dibutyryl cAMP, a membrane penetrable analog of the norepinephrine second messenger cAMP. Dibutyryl cAMP inhibited the generation of cytotoxicity at concentrations (500 μM and 10 μM) that inhibit other lymphocyte

functions (26). Such concentrations of dibutyryl cAMP appear to be physiologically relevant since lymphocytes stimulated with isoproterenol or cholera toxin contain endogenous cAMP concentrations of 11-40 pmoles cAMP/10^6 cells which we calculated as approximately 3.5-13 μM cAMP assuming an average lymphocyte diameter of 9 μm which translates into a cell volume of $3x10^{-9}$ml (29, 35-38). Our results taken together with reports of isoproterenol stimulation of cAMP production in lymphocytes (29, 35-37) are consistent with a cAMP-mediated mechanism for the catecholamine neurotransmitter-mediated inhibition of the generation of anti-MOPC-315 cytotoxicity.

However, norepinephrine inhibition was evident when added at early stages (0-2 days) in the generation of anti-MOPC-315 cytotoxicity, whereas dibutyryl cAMP inhibition was evident when added at early as well as late stages (0-4 days) in the generation of cytotoxicity suggesting that cAMP may have effects both related to and independent of norepinephrine. A number of explanations which could account for the inability of norepinephrine to downregulate later events in the generation of cytotoxicity include reduced receptor numbers and/or affinity, uncoupling of receptors to adenylyl cyclase, or use of a second cAMP signal transduction pathway(s) independent of norepinephrine.

Propranolol, a classical β-adrenergic antagonist, did not prevent norepinephrine-induced inhibition of the generation of cytotoxicity suggesting that β-adrenergic receptors may not be involved. The inability of propranolol to block the effects of

norepinephrine was unexpected since cytotoxic T-lymphocytes have β_2-adrenergic receptors (27, 28), isoproterenol binds to β_2-adrenergic receptors and stimulates cAMP production in lymphocytes (35), and as described in this report cAMP inhibited the generation of anti-tumor immunity. However, the possibilities exist that under our experimental conditions the β-adrenergic receptor may escape the antagonistic effects of propranolol or that catecholamines may act via a non-receptor-mediated mechanism, a propranolol insensitive β-adrenergic receptor, or an as yet undefined receptor, an alternative consistent with recent reports of the presence of novel neurotransmitter receptors on lymphocytes which appear to act in non-defined ways (29, 39).

The inability of propranolol to block the inhibition of the generation of anti-MOPC-315 cytotoxicity by catecholamines is similar to our previous results with parallel studies on spleen B-cell and T-cell replication, thymic cell replication and S49 T-lymphoma replication.[3] Our previous studies with the S49 mutants showed that dibutyryl cAMP but not norepinephrine inhibited proliferation by S49 mutants lacking adenylyl cyclase and that neither dibutyryl cAMP or norepinephrine inhibited proliferation by a S49 mutant lacking protein kinase A activity suggesting that norepinephrine signal transduction was primarily through an adenylyl cyclase-cAMP-protein kinase A mechanism.[3] Since receptors other than β-adrenergic receptors activate adenylyl cyclase (40-43), perhaps the norepinephrine-mediated inhibition of the generation of cytotoxicity is through a receptor which stimulates

cAMP production other than the β-adrenergic receptor. There is also the possibility that the catecholamines act via an α-adrenergic receptor. However, in our previous studies, α-adrenergic antagonists or dopaminergic antagonists did not block norepinephrine inhibition of mitogen-stimulated lymphocyte DNA synthesis.[3]

While high concentrations (100 μM) of several catecholamines inhibited the generation of anti-MOPC-315 cytotoxicity, low concentrations of norepinephrine (0.1-10 μM) enhanced (30-50%) the generation of anti-tumor cytotoxicity. An enhancement of the generation of antitumor cytotoxicity was also observed with serotonin and carbachol (a cholinergic agonist). This cholinergic agonist enhancement of the effector function of CTL's is reminiscent of a report by Strom et al. (44). In contrast to only an enhancement by carbachol and serotonin, norepinephrine exhibited a bimodal effect on the generation of antitumor cytotoxicity. A possible mechanism for the bimodal effect of norepinephrine may be that low concentrations and high concentrations of norepinephrine activate different receptors on lymphocytes. An alternative mechanism may be that high concentrations of norepinephrine stimulate the production of high levels of cAMP which inhibit the immune response whereas low concentrations of norepinephrine stimulate the production of low levels of cAMP which enhance the immune response. Consistent with this possibility are the reports that high concentrations of dibutyryl cAMP inhibit lymphocyte proliferation (45-47), whereas low concentrations of dibutyryl cAMP

stimulate lymphocyte proliferation (45).

The enhancement of the generation of cytotoxicity by norepinephrine was consistent with a recent reports by Felten et al. (2) and Hatfield et al. (48). Using a different model than that used in our studies, Felten et al. (2) and Hatfield et al. (48) showed that catecholamines augmented the generation of cytotoxicity by mouse spleen lymphocytes. Their results differ somewhat from those reported here since they reported that a 100 μM concentration of norepinephrine increased the generation of cytotoxicity whereas we find that this concentration was inhibitory and that 0.1-1 μM norepinephrine concentrations elevated the generation of cytotoxicity (48). The exact reasons for these differences are not known. However, Hatfield et al. (48) and Felten et al. (2) examined the effect of norepinephrine on the generation of an allogeneic response and the studies reported here examined the effect of norepinephrine on the generation of a syngeneic response and it is possible that the sensitivity to norepinephrine regulation of allogeneic responses differ from those for regulation of syngeneic responses. Differences in regulation of the generation of syngeneic and allogeneic CTL responses have been reported with regards to interleukin 4 requirements (49).

In this report, we have demonstrated that high concentrations of catecholamines can inhibit the generation of anti-MOPC-315 cytotoxicity. The effect of catecholamines is not limited to inhibition of the generation of antitumor cytotoxicity. In fact, catecholamines can inhibit expression of the lytic activity by

fully activated CTL's. Henney et al. (50) showed that the secretory phase of target cell lysis is inhibited by isoproterenol (0.1-100 μM) and cAMP (500 μM). Therefore, catecholamines inhibit both initial events in the generation of cytotoxic activity, as described herein, and degranulation of CTL's (50). The catecholamine inhibition of the generation and expression of CTL activity *in vitro* suggests that stress-induced elevation of catecholamine levels may inhibit tumor eradication *in vivo* by down regulating the generation of CTL activity as well as by down regulating the delivery of the lethal hit by the CTL's. Our studies describing the inhibition of antitumor cytotoxicity supply a framework for further studies of the mechanisms for stress-induced modulation of the generation of antitumor immunity.

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Figure Legends

Figure 1. Concentration Curves for Catecholamine Modulation of the In Vitro Generation of Anti-MOPC-315 Cytotoxicity by BALB/c Spleen Cells. Norepinephrine (*closed triangle, dashed line*), epinephrine (*closed square, dotted line*), isoproterenol (*closed diamond, dashed/dotted line*), or the HCl solvent control (*closed circle, solid line*) was added to normal BALB/c spleen cells admixed with mitomycin-C-treated syngeneic MOPC-315 plasmacytoma cells for 5 days. The in vitro stimulated cells were evaluated for their lytic activity at effector/target cell (E/T) ratios of (A) 200:1, (B) 100:1, and (C) 50:1. Data are from a representative experiment of three experiments. Where error bars are not shown, the error bars are smaller than the symbol. *, Statistical significance ($p<0.05$) relative to lytic activity exhibited by spleen cells stimulated with MOPC-315 cells in the absence of agonists.

Figure 2. Concentration Curves for Modulation of the Generation of Anti-MOPC-315 Cytotoxicity by Dopamine, Carbachol, and Serotonin. Dopamine (*closed triangle, dashed line*), serotonin (*closed square, dotted line*), carbachol (*closed diamond, dashed/dotted line*) or HCl (*closed circle, solid line*) was added with normal BALB/c spleen cells admixed with mitomycin-C-treated syngeneic MOPC-315 plasmacytoma cells for 5 days. The in vitro immunized cells were

evaluated for their lytic activity at E/T ratios of (A) 200:1, (B) 100:1, and (C) 50:1. Data are from the same representative experiment shown in figure 1. Where error bars are not shown, the error bars are smaller than the symbol. *, Statistical significance ($p<0.05$) relative to lytic activity of spleen cells incubated with mitomycin-C-treated MOPC-315 cells.

Figure 3. Concentration Curve for Dibutyryl cAMP Inhibition of the Generation of Anti-MOPC-315 Cytotoxicity. Dibutyryl cAMP was incubated with normal BALB/c spleen cells admixed with mitomycin-C-treated syngeneic MOPC-315 plasmacytoma cells for 5 days followed by assessment of lytic activity. The data shown are the lytic activity of in vitro immunized cells at an effector/target cell ratio of 100:1. Data are from a representative experiment of more than three experiments. *, Statistical significance ($p<0.05$) relative to lytic activity of spleen cells incubated with mitomycin-C-treated MOPC-315 cells.

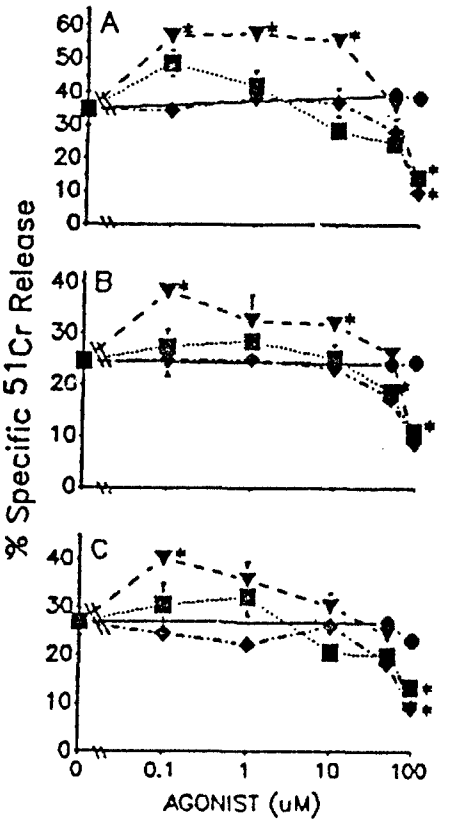
Figure 4. Extent of Inhibition of Anti-MOPC-315 Cytotoxicity by Norepinephrine and Dibutyryl cAMP after 3, 4, or 5 days. Norepinephrine (NE), dibutyryl cAMP (DBcAMP), or the HCl solvent control was incubated with normal BALB/c spleen cells admixed with mitomycin-C-treated MOPC-315 cells for 3, 4, or 5 days followed by assessment of lytic activity. The data shown are the percent inhibition of lytic activity of in vitro immunized cells at an E/T ratio of

100:1. Data are from a representative experiment of two experiments. *, Statistical significance ($p<0.05$) relative to the percent inhibition of lytic activity of spleen cells stimulated mitomycin-C-treated MOPC-315 cells in the presence of HCl (100 μM). The % inhibition by 50 μM NE was significantly greater on day 4 as compared to the % inhibition on day 3 and 5 ($p<0.05$). For the representative experiment shown in figure 4, spleen cells stimulated in vitro with MOPC-315 cells in the presence of HCl led to a % specific ^{51}Cr release of 18±1, 48±1, and 39±4 on days 3, 4, and 5, respectively.

Figure 5. Effect of Temporal Addition of Norepinephrine and Dibutyryl cAMP on the Generation of Anti-MOPC-315 Cytotoxicity. Spleen cells from BALB/c mice were cocultured with mitomycin-C-treated MOPC-315 cells. On days 0-4 after culture initiation, norepinephrine (100 μM, *closed diamond, dashed/dotted line*), dibutyryl cAMP (500 μM, *open diamond, dashed line*), or the HCl solvent control (*closed circle, solid line*) were added to the cultures. On day 5, the in vitro immunized cells were evaluated for their lytic activity at effector/target cell ratio of (A) 100:1 and (B) 25:1. Data are from the same representative experiment shown in figure 4. Where error bars are not shown, the error bars are smaller than the symbol. *, Statistical significance relative to lytic activity of spleen cells incubated with HCl (100 μM) and

mitomycin-C-treated MOPC-315 cells.

Figure 6. Effect of Propranolol on Norepinephrine Inhibition of the Generation of Anti-MOPC-315 Cytotoxicity. Spleen cells from BALB/c mice were incubated with propranolol for 30 minutes prior to addition of norepinephrine at 50 μM (*closed square, dotted line*), norepinephrine at 100 μM (*closed triangle, dashed line*), and mitomycin-C-treated MOPC-315 cells. Controls (*closed circle, solid line*) consisted of cells incubated with mitomycin-C-treated MOPC-315 cells in the absence of norepinephrine. After 5 days, the in vitro immunized cells were evaluated for their lytic activity. Data shown are for an E/T ratio of 100:1. Data are from a representative experiment of two experiments. Where error bars are not shown, the error bars are smaller than the symbol.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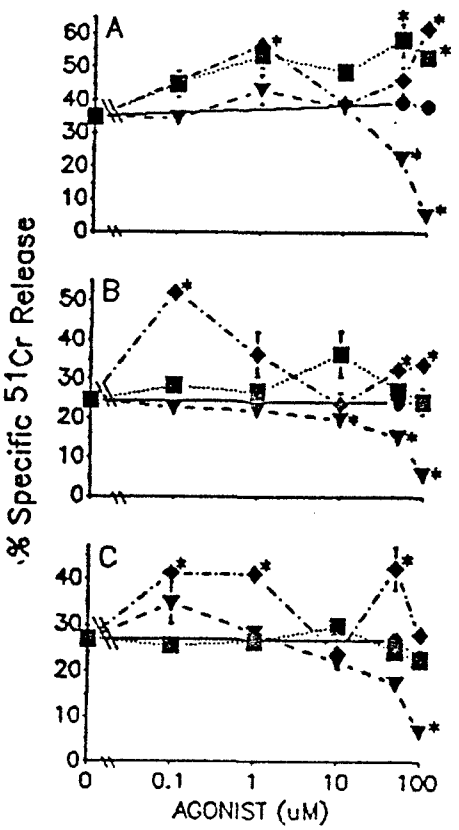
100
% Specific 51Cr Release
AGONIST (uM)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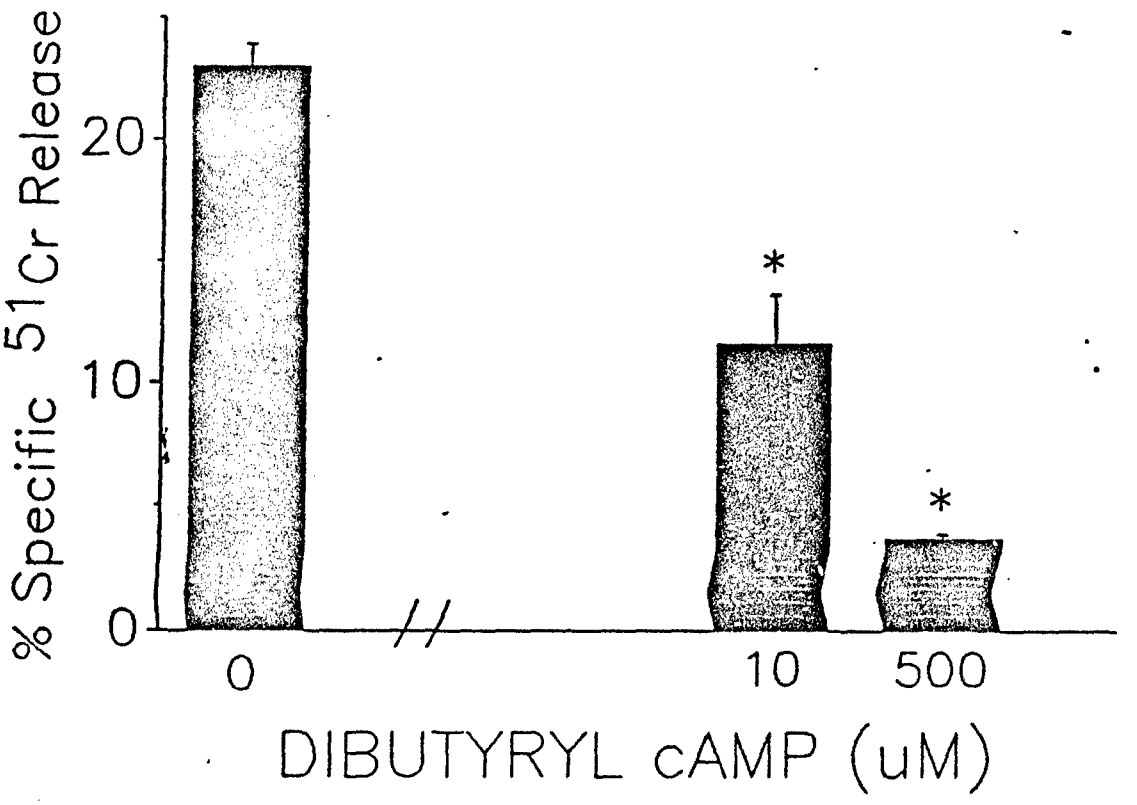
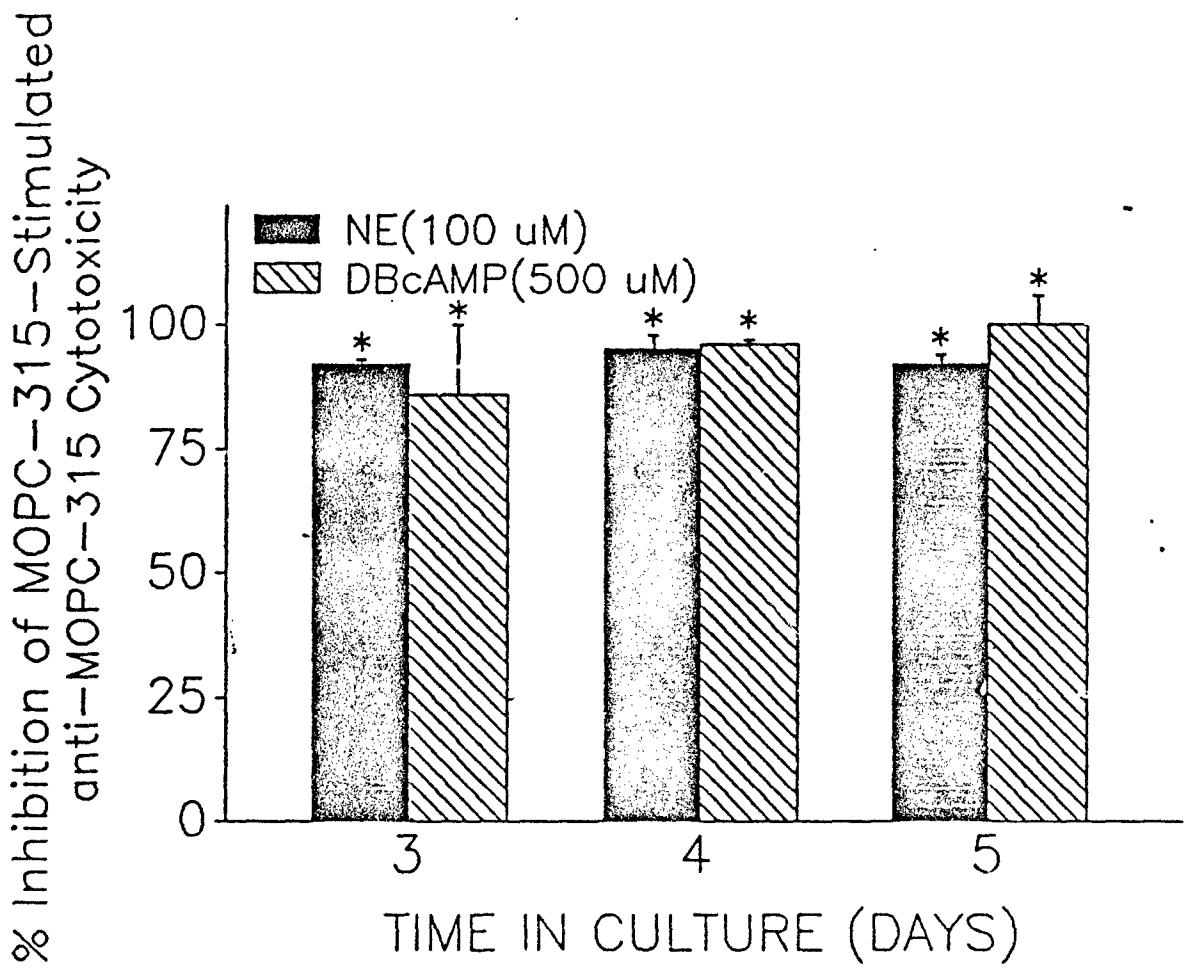
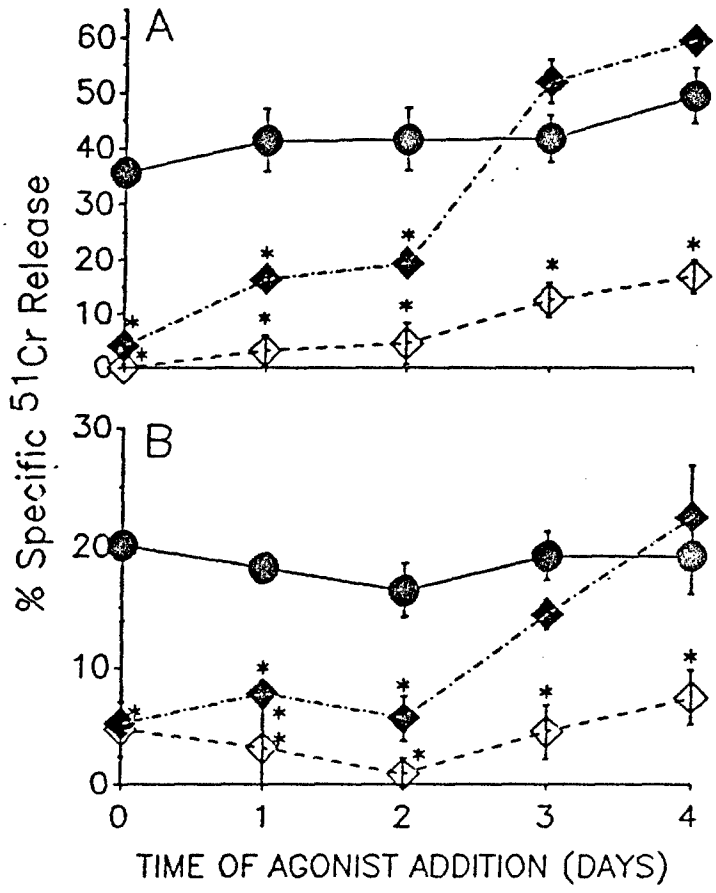
% Specific 51Cr Release
AGONIST (uM)


% Specific 51Cr Release
20
10
0
*
*
0
10
500
DIBUTYRYL cAMP (uM)


% Inhibition of MOPC-315-Stimulated anti-MOPC-315 Cytotoxicity
NE(100 uM)
DBcAMP(500 uM)
100
75
50
25
0
3
4
5
TIME IN CULTURE (DAYS)


A
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30
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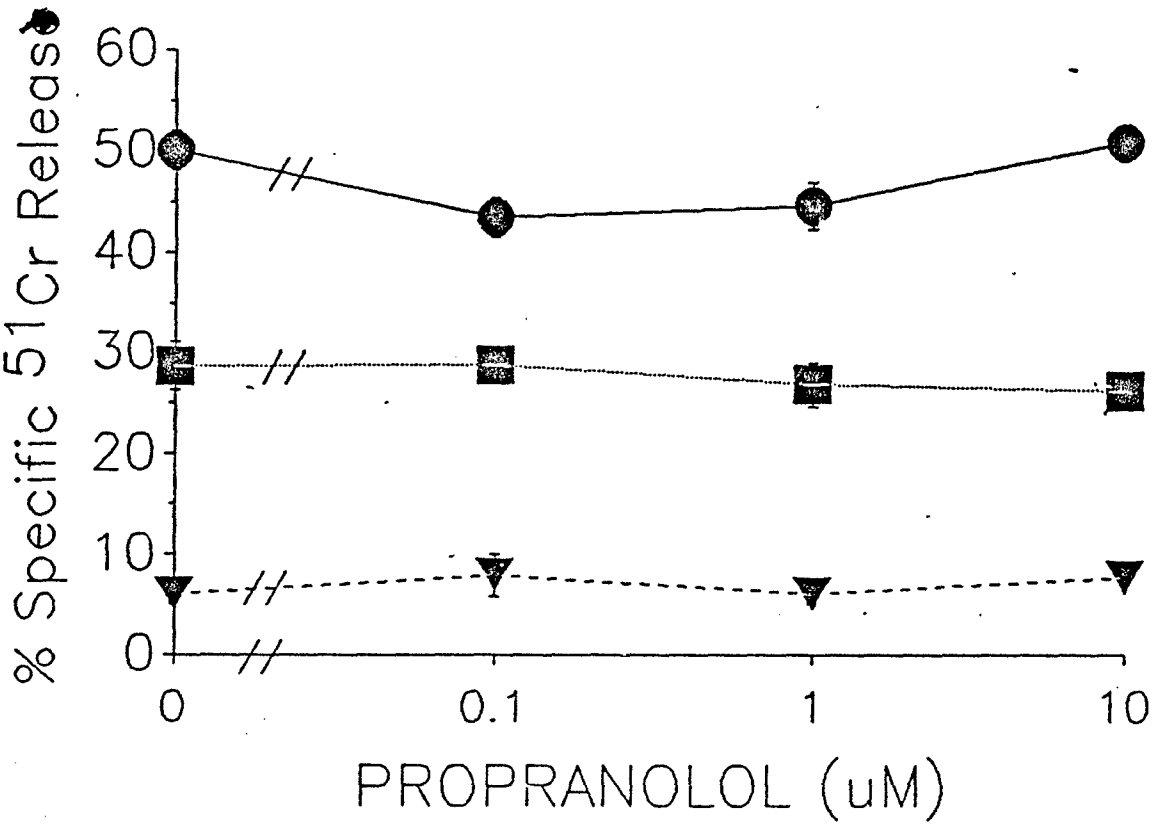
2
3
4
% Specific 51Cr Release
TIME OF AGONIST ADDITION (DAYS)


% Specific 51Cr Release
60
50
40
30
20
10
0
0
0.1
1
10
PROPRANOLOL (uM)