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Modulation of Macrophage Chemiluminescence by Biological Response Modifiers and Neuroendocrine Hormones

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MODULATION OF MACROPHAGE CHEMILUMINESCENCE BY BIOLOGICAL RESPONSE MODIFIERS AND NEUROENDOCRINE HORMONES

by

Jeffrey M. Tosk

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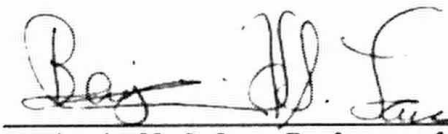

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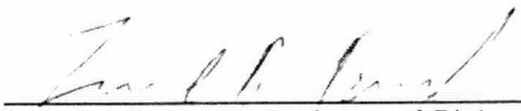

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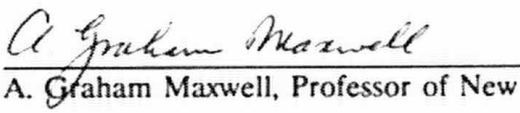

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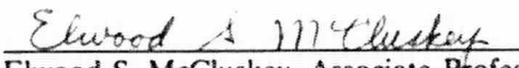

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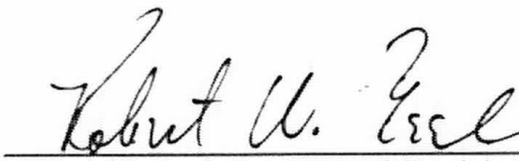

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INTRODUCTION

One of the earliest observations that psychogenic parameters could influence the immune response was made in 1918 [1]. Other sporadic reports alluding to CNS interaction with immunity can be found in the literature from the first half of this century, and several interesting reviews place psychoneuroimmunology in an historical perspective [2].

Of the many factors suggesting a neuro-immune regulatory axis, the effects of stress on the immune response have provided the most convincing evidence for such a network. A variety of paradigms have been employed to model the immunological consequences of stress in humans. These include the evaluation of immune function during forced wakefullness, bereavement, divorce and unemployment [3]. In experimental animals stress has an adverse impact on such important immune parameters as the rate and quantity of Ig production, T-lymphocyte response to mitogenic challenge, T cell cytotoxic activity, natural killer (NK) cell function and host-graft relationships. This body of literature has been extensively reviewed [4].

Psychoneuroimmunology of Macrophages

A variety of studies have demonstrated that neuroendocrine hormones participate in macrophage activity. UK-14304 and norepinephrine, both alpha 2-adrenergic agonists, can augment LPS-stimulated tumor necrosis factor (TNF)

from elicited macrophages [5]. The differentiation of the human histiocytic lymphoma cell line U 937 into macrophage-like cells by phorbol ester is inhibited by cortisol [6]. Epinephrine induces a dose-dependent decrease in macrophage spreading [7].

There are several reports that numerous activities of phagocytic cells are modulated by neuropeptides and neuroendocrine hormones [8-12]. ß-Adrenergic agonists were reported by Bourne et al. [13] and Cox and Karnofsky [14] to inhibit phagocyte chemotaxis, bactericidal activity, enzyme release and superoxide anion generation. More recently, Nielson [15] reported that the ß-adrenergic agonist isoproterenol inhibits the respiratory burst of phagocytes in a manner dependent upon the mechanism of cell activation. Koff and Dunegan [16] observed an inverted-U dose response to norepinephrine with respect to its effects on macrophage-mediated cytotoxicity when cells were activated with γ-interferon. At 10^{-10} M and 10^{-8} M in norepinephrine a significant enhancement of cytotoxicity towards tumor cell targets was noted, while higher dosages were associated with inhibition of cytolysis. These authors also reported that the neuropeptide neurotensin enhanced the cytolytic activity of γ-INF-activated macrophages, while substance P, α-endorphin, ß-endorphin, Leu-enkephalin and Met-enkephalin had no effect. Foris, Medgyesi and Hauck [17] reported that met-enkephalin exerted a bidirectional effect on macrophage effector function which was naloxone reversible at low levels of met-ENK. Foris, et al. [18,19] have also reported that met-ENK can induce a significant increase in the

luminol-dependant chemiluminescence of thiogycollate-elicited rat peritoneal macrophages. Foster and Moore [20] found that the opioid peptides dynorphin A and related fragments significantly enhanced the ability of murine peritoneal macrophages previously activated with mixed α + ß-INF and lipopolysaccharide (LPS) to lyse P815 mastocytoma target cells; an effect which was naloxone-reversible.

Simpkins, Dickey and Fink [21] and van Epps and Saland [22] have shown that leukocyte chemotaxis is enhanced by ß-endorphin. Sharp et al [23] showed that opioid peptides themselves could act as oxidative burst stimulants in phagocytes.

By applying a novel methodology based on the molecular recognition hypothesis, Carr and Blalock [24] isolated and purified receptors shared by the immune and neuroendocrine systems. Biochemical analysis revealed them to be virtually identical with respect to their physicochemical and functional properties. Thus, bidirectional communication between the immune and neuroendocrine systems seems to result from a common set of hormones and receptors which are shared by the two systems. Collectively, these studies provide convincing evidence of important functional interactions between the neuroendocrine and immune systems.

Few studies have been undertaken to determine the cellular and physiological mechanisms through which the CNS might influence immunity. This may be due in part to the lack of suitable models for mechanistic research

in this area. Most of the aforementioned studies have employed whole animals or immune cells derived from blood and other tissue. Under these conditions it becomes difficult to attribute a result to one specific cell type, no less determine the subcellular mechanisms which account for a given observation. Furthermore, the cost and concern over animal experimentation may shortly have a chilling effect on this approach.

Although the study findings reviewed above are consistent with the hypothesis that neuro-immune interaction is involved in the regulation of the immune response, animal and human studies have been unable to establish the cellular mechanisms needed to explain the detailed function of neuro-endocrine-immune regulatory axis. Considerable biological variation associated with human and animal experimentation involving behavioral variables severely limit the ability of these approaches to resolve major questions raised by psychoneuroimmunologists. Indeed, if one believed, for example, that endorphins and/or biogenic amines mediate the effects of stress on any given immune parameter, an investigator would be loathe to administer opioid, dopaminergic, adrenergic and noradrenergic antagonist to controls in a human study in spite of the unique ability of such treatments to clarify these questions. It was therefore a major goal of the studies reported in this dissertation to develop an in vitro model which could circumvent these problems and restrictions while providing a highly reproducible and relevant system in which neuroimmune interaction could be studied at the cellular level. The

development of the model reported here accepted the premise that an immortal cell line isolated from a malignancy primary to an immunocyte could possess such useful attributes as immortality. A tumor cell line is competent to enter into cell division without the restrictions observed in non-neoplastic cells. Thus a single neoplastic cell can give rise to a large and virtually limitless number of "progeny" which possess a manageable amount of clonal variation.

An additional attribute of tumor cell lines is their retention of sufficient cytogenetic similarity to the precursor immunocyte that the immortal cell line will appear in many regards to behave much like a normal immunocyte. In practice this attribute is conveniently determined by examining the cell line for appropriate receptors (eg. immunoglobulin receptors), the secretion of cell-specific cytokines (eg. IL-1 and TNF secretion by a macrophage cell line) as well as such functional characteristics as cell-specific lysis of generally accepted targets.

It must be carefully noted that like any model, the use of an immortal immunocyte cell line for studying neuroimmune interaction, or any other properties of the immune system is not without its limitations. For example, the normal immunocyte does not exhibit the aggressive growth associated with a tumor cell line. Furthermore, while the tumor cell derived from an immunocyte may indeed produce an immunologically correct cytokine, the normal genetic regulation of cytokine expression may be modified or virtually nonexistent. Receptor densities may be, and probably are, different than those encountered

in a normal immunocyte. Nevertheless, the convenience, cost-effectiveness and general similarity of an immortal cell line to its normal counterpart are well known and have convinced many investigators to employ them in their research [atcc]. In practice, the ATCC Catalog of Cell Lines is a valuable research tool in itself, allowing the investigator to rapidly survey the most commonly used and immediately available cell lines and appraise their potential usefulness for a given application.

Excited Electronic States in Biological Systems

Having had a long-standing interest in the role of excited electronic states in biological systems [25], the author sought to develop a cellular model applicable to the study of neuroendocrine regulation of oxidative burst in macrophages. The events which contribute to oxidative burst (OB) are believed to involve a variety of molecular excitations in which molecular oxygen is an integral participant. Indeed, the process of OB involves the generation of numerous reactive oxygen species (ROS) which include superoxide anion, singlet oxygen, hydroxyl free radicals and hydrogen peroxide [26]. Additional excited states result from the interaction of this redox matrix with ecosinoids and related peroxidizable lipids [27]. ROS production during OB is among those relatively rare biological events heralded by the emission of photons in a process referred to as chemiluminescence and occasionally as bioluminescence. In native OB a small but significant photon flux was first observed by Steel's group in 1975 [28]

using alveolar macrophages derived from the rabbit. Shortly thereafter the same group reported that the attending chemiluminescence could be enhanced or amplified by the addition of luminol, a hydrazide which reacts with peroxides and to a lesser extent other ROS by emitting photons during the relaxation of an electronically excited aldehyde moiety [29].

Reactive oxygen species and the antitumor macrophage

The generation of reactive oxygen species (ROS) by neutrophils [30-35] and macrophages [36,37-39] represents a major, but by no means exclusive mechanism by which these cells mediate their antimicrobial and tumoricidal functions [40,41]. Much of what is known about the induction and release of these species has been elucidated using freshly isolated cells from blood and other tissues of experimental animals.

The decomposition of ROS is accompanied by the emission of light during a process referred to as chemiluminescence (CL) [28,42]. Addition of the exogenous substrate luminol facilitates the detection of the attending CL [29]. CL has been employed as a means of studying factors affecting the oxidative burst of monocytes and macrophages [43-45].

While the ultimate significance of ROS production during OB remains to be elucidated, these species (hydrogen peroxide in particular) possess potent cytotoxic activity which is believed to play a role in the ability of macrophages to kill microorganisms. Since macrophages are involved in the host response to

malignancy the role of ROS has been suggested to extend to the killing of tumor cells as well [38].

A few studies have examined the CL accompanying the oxidative burst in some monocyte-macrophage cell lines. Stendahl, Dahlgren and Hed [46] reported CL accompanied oxidative metabolism in the human promyelocytic cell line HL-60 following differentiation by dimethyl sulfoxide. CL associated with oxidative burst has subsequently been observed during oxidative burst as a marker for differentiation in HL-60 cells [47,48]. CL associated with oxidative burst has also been reported in the human monocytic cell line U937 following cellular differentiation [49,50]. Zavala and Lenfant [45] have studied factors affecting CL during arachidonic acid-stimulated respiratory burst in the murine macrophage-like P338D_1 cell line. CL has also been reported in the murine C4Mϕ macrophage cell line [51].

It was therefore an important goal of the present research to develop an entirely in vitro model using an immortal cell line for investigating the influence of neuroendocrine factors on the immune response.

The first chapter in this dissertation describes the use of a murine macrophage cell line, J774 as a model for macrophage-mediated antitumor immunity. These cells are shown to exhibit a light-emitting (chemiluminescent) oxidative burst which is conveniently quantitated. The oxidative burst is modulated by a variety of immunotherapeutic agents under conditions which similarly affect the ability of J774 cells to kill tumor cell targets. The second

chapter considers the application of the J774 macrophage model for the study of neuroendocrine modulation of the oxidative burst. It is shown that the chemiluminescence attending oxidative burst is enhanced or inhibited in a dose-dependent fashion by incubation of J774 cells with neuropeptides and neuroendocrine hormones over a range of dosages which includes their normal physiological concentrations. Such substances have been thought to mediate the neuroendocrine modulation of the immune response. In the case of the neurotransmitter norepinephrine, a mechanistic experiment determined that this substance exerted an effect on the membrane potential of J774 macrophages similar to its effects on neuronal cells.

It is anticipated that the model described here will be of continuing value to investigators studying the interaction of the CNS with the immune response against cancer at the cellular and molecular levels.

A substantial body of evidence has recently emerged supporting the hypothesis that a neuro-endocrine-immune regulatory axis exists which effects feedback control of the immune response [52,53]. Among the observations in support of this paradigm are reports that neuropeptides and neuroendocrine hormones can influence cells of both the lymphocytic arm of the immune system and the mononuclear phagocyte system. Stress related hormones such as adrenocortitropin, norepinephrine, epinephrine and glucocorticoids [54,55] suppress a wide range of T cell responses including lymphokine production. In the case of ß-adrenergic substances, possibly through the down-regulation of

interleukin 2 (IL-2) receptors [56]. Endogenous opioid peptides such as endorphins and enkephalins affect the response of T cells to mitogenic stimulation [57,58] and modulate the cytotoxic activity of natural killer (NK) cells [59]. Opioid peptides may also influence T cell response through a modification of the early calcium influx accompanying lymphocyte activation [60].

Cells of the mononuclear phagocyte system play a prominent role in the host defense against neoplastic disease, possibly through the generation of cytotoxic reactive oxygen species [61]. When stimulated by immunomodulatory substances exemplified by γ-interferon (γ-INF) [62,63], lipopolysaccharide (LPS), and the combination of LPS and α + ß interferon (α + ß-INF), macrophages develop cytotoxic activity characterized by the selective binding and destruction f tumor cells [64,65].

The generation of reactive oxygen species (ROS) by macrophages is believed to account for at least part of their antimicrobial and tumoricidal activity [61,66-68]. The decomposition of these species, which include hydrogen peroxide and superoxide anion, is accompanied by the emission of light during a process referred to as chemiluminescence (CL) [69]. Addition of the exogenous substrate luminol facilitates the detection of the attending CL [27]. CL has been used to study factors affecting the oxidative burst of monocytes and macrophages [70]. I have recently reported that the J774 murine macrophage cell line exhibits a chemiluminescent oxidative burst which is modulated by biological response

modifiers such as *Corynebacterium parvum* (CP), bacillus Calmette-Guerin (BCG), LPS alone and in combination with α + ß INF [56]. I now report that the CL emission of J774 cells can be modulated by the opioid peptides ß-endorphin, dynorphin A, dynorphin fragment 1-13 and the neuropeptides neurotensin and tuftsin. The ß-adrenergic agonist norepinephrine also modulated the CL. The inhibitory neurotransmitter γ-aminobutyric acid and its pharmacological probe, diazepam, likewise modulated J774 CL emission, as did the pineal hormone melatonin. Norepinephrine induced a transient hyperpolarization of membrane potential in an adherent population of J774 macrophages.

CHAPTER 1

MATERIALS AND METHODS

Reagents

Phenol-extracted lipopolysaccharide (LPS) from *E. coli* 0111:B4 and sodium dodecyl sulfate (Sigma, St. Louis, MO); formalin killed *C. parvum* (CP) (Burroughs-Wellcome, Research Triangle Park, NC); BCG vaccine, USP (Bionetics Research, Inc., Rockville, MD); mouse interferon α + ß (Lee Biomolecular Research Inc., San Diego, CA); and [^{51}Cr]chromium ($Na_2{}^{51}CrO_4$), specific activity 446.46 mCi/mg (New England Nuclear, Boston, MA).

Cell Lines

The murine macrophage cell line J774A.1 was obtained from the American Type Culture Collection (Rockville, MD). This cell line has been described as a reticulum cell sarcoma by Ralph et al. [71-73]. J774 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Grand Island, NY) supplemented with 10% heat inactivated fetal bovine serum (FBS), penicillin (50 U/ml) and streptomycin (250 mg/ml). The viability of cells used in experiments was always >90% as determined by trypan blue exclusion.

The P815 murine mastocytoma cell line was kindly provided by Dr. J.L. Pace, University of Florida, Gainesville, and served as the tumor cell target in cytotoxicity assays. P815 cells were maintained in modified minimal essential

Eagles medium (H-MEM) (GIBCO) with 10% FBS, HEPES (15 mM) (Sigma) and antibiotics. Cell lines were maintained at 37° C in a humidified atmosphere of 5% CO_2 and were determined to be free of mycoplasma contamination.

Chemiluminescence (CL) Assays

The CL response of J774 cells was assayed at 37° C in an automated luminometer (Picolite model 6500, Packard Instrument Co., Downers Grove, IL) interfaced with a PC-compatible computer. Luminometry experiments were performed in triplicate. Mixtures of cells and BRMs were made up in DME/F-12 medium without indicator (Sigma), supplemented with 10% FBS and antibiotics. These suspensions were added to 55 X 12 mm plastic luminometry tubes (Los Alamos Diagnostics, Los Alamos, NM). Each tube contained 2-5 X 10^5 cells in a volume of one ml. Except for time-course experiments, cell suspensions for luminometry were incubated with BRMs at 37° C in 5% CO_2 for 18 h prior to the assay to allow the cells to adhere to the bottom of the luminometry tubes. After incubation, 800 l of cell-free medium in each tube were removed and discarded. Repeated examination showed discarded medium contained <0.1% of the original number of cells plated. The CL assay was initiated by the automatic addition of 200 l of an opsonized zymosan A suspension containing luminol (ZAP, Los Alamos Diagnostics, Los Alamos, NM). The photon emission from samples was counted every 4 min for 36 min. The area under curves representing light emission over time was calculated and

reported as integrated photon emission.

Assay for J774-mediated cytolytic activity

Killing of labeled P815 mastocytoma cells was determined in a 16-h ^{51}Cr release assay for macrophage-mediated cytotoxicity [74]. Briefly, target cells were prelabeled for 1 h at 37°C with ^{51}Cr (300 Ci/10^7 cells), and washed. J774 monolayers were prepared in 96-well microtiter plates by plating cells in 100 l 8H-MEM + 10% FBS. After a brief centrifugation, ^{51}Cr-labeled P815 cells in 100 l H-MEM were seeded into each well. An effector:target ratio of 50:1 was used. Aliquots of BRMs were then added. Spontaneous release of label was determined from wells containing only labeled P815 cells. Maximum release was determined from wells containing labeled cells and 10% sodium dodecyl sulfate. After a 16 h incubation at 37°C in 5% CO_2, 100 l of supernatant in each well were removed and assayed for radioactivity. Experiments were performed in triplicate. Results were expressed as % ^{51}Cr-release, calculated as described previously [75].

Statistical Analysis

Where appropriate, data were expressed as the mean ± SEM. For multiple group comparisons, one-way analysis of variance (ANOVA) was employed followed by Tukey's test for HSD (honestly significant difference). Statistical significance was defined as $p < 0.05$. The statistical procedures were performed with Statgraphics software version 2.1 (STSC, Inc., Rockville, MD).

RESULTS

Initial experiments indicated that J774 cells have a strict requirement in that chemiluminescence can be observed only when these cells are allowed to adhere to the bottom of the plastic luminometry tubes. Suspensions of these cells failed to exhibit luminescence. The CL of J774 cells triggered by zymosan was in the order of 10^6-10^7 counts, integrated over a period of 36 min, only slightly lower in magnitude than freshly isolated macrophages from mice (data not shown).

Fig. 1 illustrates the CL response of J774 cells incubated with various dosages of CP (1.4-140 g/ml) for 18 h prior to the assay triggered by zymosan. Optimal CL was observed with pre-incubation using 14 g/ml of CP. Dosages above this level were associated with a loss of CL. Fig. 2 illustrates the effect of incubation time on CL using the optimal 14 g/ml dosage of CP. The CP-modulated CL continued to increase throughout the course of the 44 h experiment. The CL levels of untreated cells were significantly below those of CP-pretreated and peaked at 30 h of incubation.

Fig. 3 shows the effect of BCG on J774 CL following 18 h of incubation. Maximal CL occurred with 5×10^4 cfu/ml. Dosages above 8×10^5 cfu/ml depressed CL activity. Fig. 4 shows the effect of LPS on the CL of J774 cells. The cells responded to as little as 0.5 ng LPS/ml. A plateau in activity was observed at dosages from 5 to 50 ng/ml.

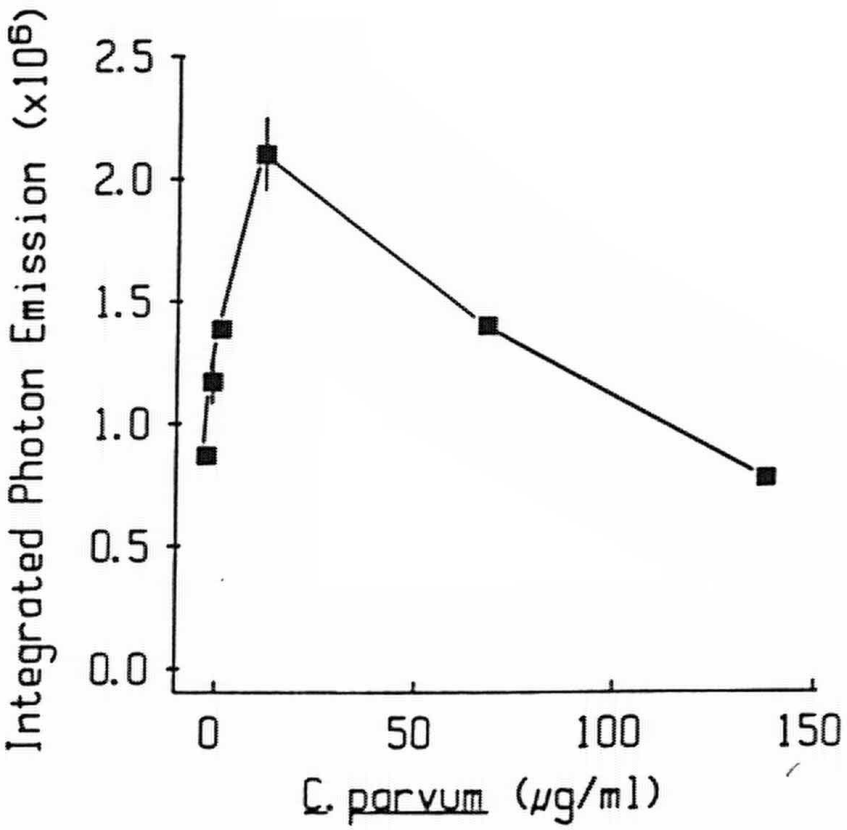

Figure 1. Effect of CP dosage on CL of J774 cells. Cells were preincubated with CP for 18 h prior to CL assay. Points represent the mean ± SEM (if SEM does not show, it is smaller than the symbol).

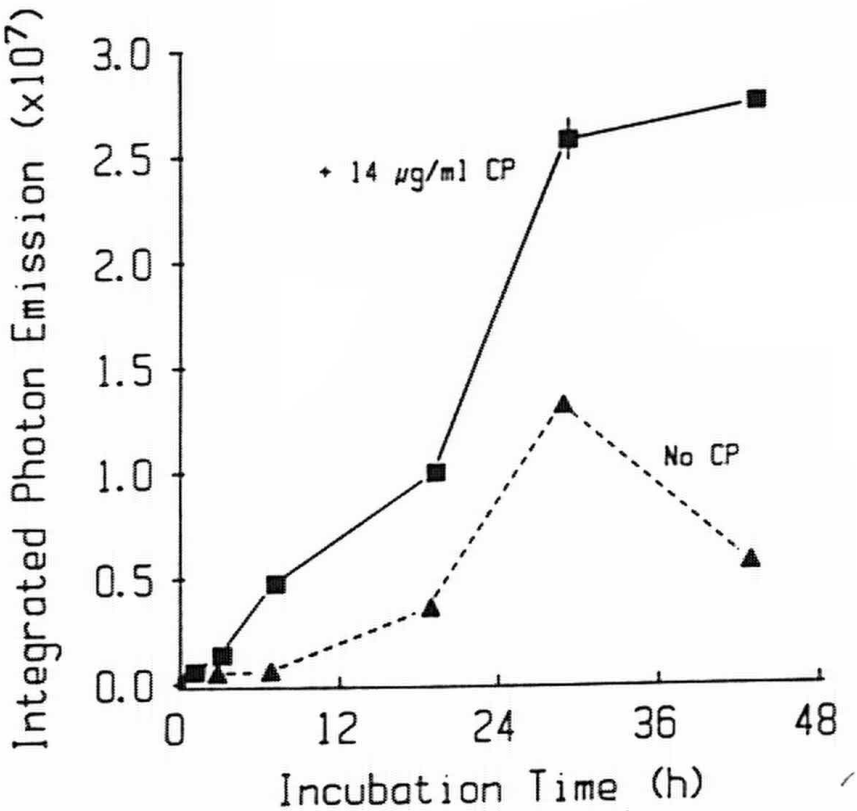

Figure 2. Effect of preincubation time on CP-stimulated CL of J774 cells. Cells were incubated with 14 µg/ml CP (■——■), or without CP (▲- - -▲).

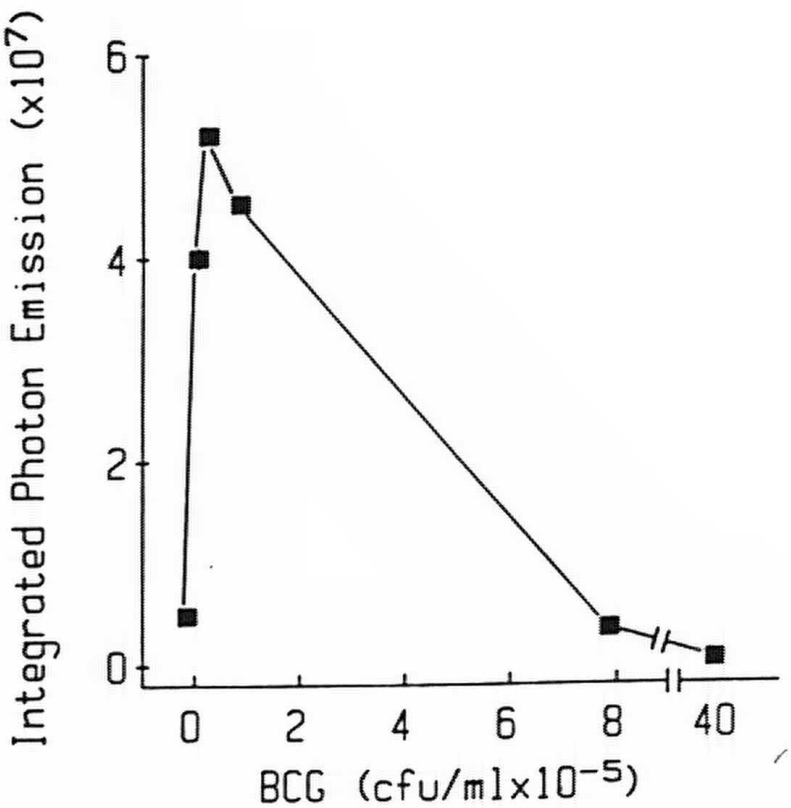

Figure 3. Effect of BCG dosage on CL of J774 cells. Cells were preincubated with BCG for 18 h prior to CL assay.

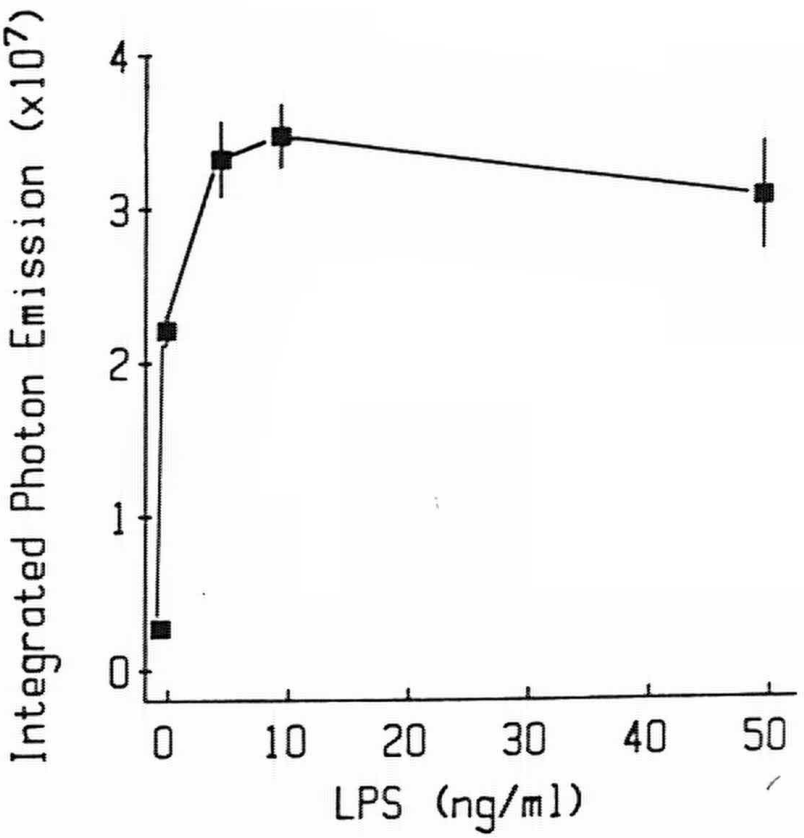

Figure 4. Effect of LPS dosage on CL of J774 cells. Cells were preincubated with LPS for 18 h prior to CL assay.

Fig. 5 shows the effect of murine interferon α + ß (INF) on the ability of LPS to stimulate CL in J774 cells. An INF dosage of 440 U/ml had no effect on CL. LPS alone (5 ng/ml) stimulated CL. Co-incubation of J774 cells with INF and LPS further increased the level of CL. A similar effect resulting from the interaction of INF with CP is illustrated in Fig. 6. In this experiment 14 g/ml of CP was substituted for the LPS. CP alone enhanced the CL of J774 cells while INF had no effect. Co-incubation of J774 cells with CP and INF exhibited a synergistic enhancement of CL.

Table 1 summarizes the results of experiments demonstrating the effects of CP or LPS on J774-mediated cytotoxicity. Untreated J774 cells exhibited a low but significant level of lytic activity toward P815 mastocytoma targets. CP enhanced this activity in a dose-dependent manner. Similarly, LPS was observed to augment J774-mediated cytotoxicity.

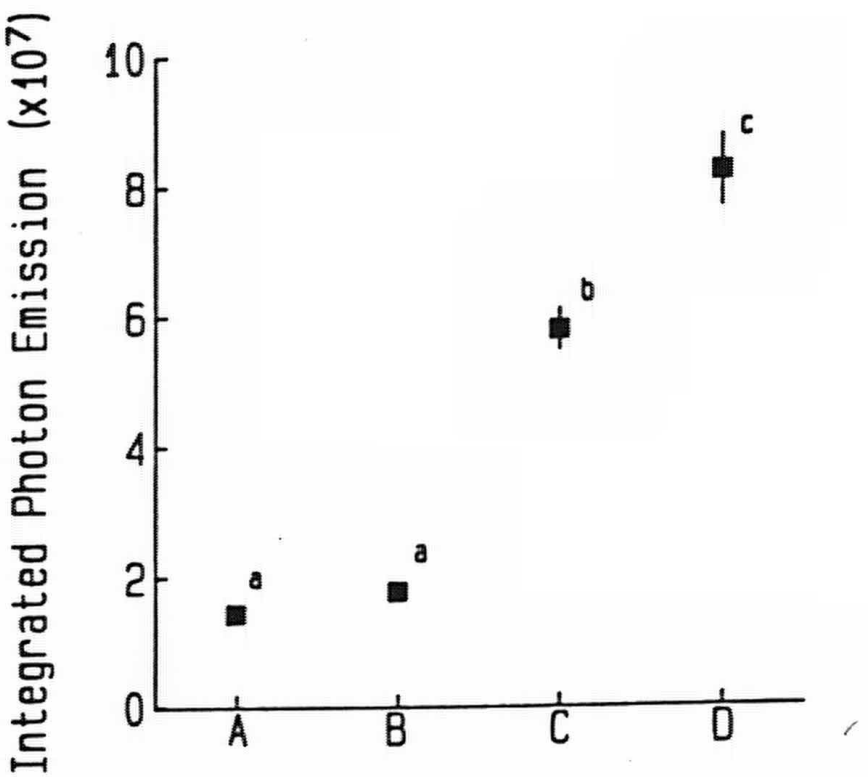

Figure 5. Effect of murine interferon α+β (INF) (440 U/ml) on LPS- (5 ng/ml) stimulated CL in J774 cells. A = cells alone, B = cells + INF, C = cells + LPS, D = cells + LPS and INF. Cells were preincubated with INF, LPS or both reagents for 18 h prior to CL assay. Tukey's HSD test demonstrated three statistically different responses (a, b, and c) at $p < 0.05$, e.g. INF alone had no effect. LPS alone did enhance CL; and co-incubation with both INF and LPS further enhanced CL.

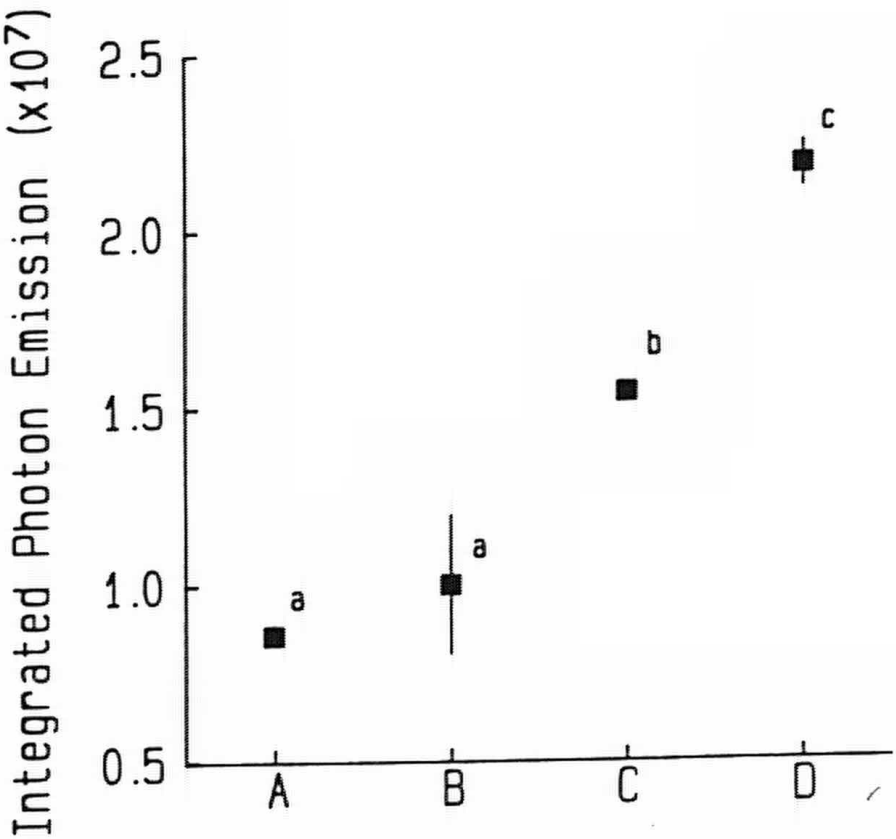

Figure 6. Effect of murine interferon α+β (INF) (440 U/ml) on CP- (14 μg/ml) stimulated CL in J774 cells. A = cells alone, B = cells + INF, C = cells + CP, D = cells + CP and INF. Cells were preincubated with INF, CP or both reagents for 18 h prior to CL assay. Tukey's HSD test demonstrated three statistically different responses (a, b and c) at $p < 0.05$; - INF alone had no effect. CP alone enhanced CL while co-incubation with INF and CP further enhance CL.

Table 1

Effect of CP and LPS on J774-mediated cytotoxicity[†]

Treatment	% ^{51}Cr Release
Untreated Cells	7.0 ± 0.34
CP 14 μg/well	17.9 ± 0.78
CP 35 μg/well	36.3 ± 0.94
CP 70 μg/well	51.3 ± 1.25
LPS 5 ng/well	26.1 ± 1.15
LPS 10 ng/well	34.1 ± 1.35

[†]Assayed at an E:T ratio of 50:1. Data represent the mean ± SEM.

DISCUSSION

Macrophages have been implicated as a major class of effectors in the host response to neoplasia [77,77,39,78]. Cells of the monocyte-macrophage lineage are known to exhibit tumoricidal activity following stimulation by γ-interferon [79,80], CP [82], BCG [76], and bacterial products such as muramyl dipeptide [83] and LPS [83]. While the mechanisms involved remain obscure, the generation of ROS by activated macrophages is considered a major participant in mediating the tumor-lytic effect [36-39]. I investigated the J774 cell line in an attempt to overcome problems associated with studies employing partially purified macrophages. J774 cells exhibit adherence, macrophage-like morphology, receptors for immunoglobulin, and are able to lyse target cells [83]. They synthesize interleukin 1 [84], and release tumor necrosis factor and lysosomal enzymes upon stimulation with LPS [85]. Clones of the J774 cell line are capable of oxidizing glucose by the hexose monophosphate shunt while generating superoxide and hydrogen peroxide [86]. In light of these accounts it is surprising that chemiluminescence associated with the oxidative burst of J774 cells has not previously been reported. De Baetselier and Schram [43] have described a J774 clone designated J774-C2E2-HAT which they employed for polyethylene glycol-mediated somatic hybridization with normal murine macrophages. The resulting macrophage hybridomas exhibit a chemiluminescent oxidative burst among other macrophage-like characteristics which were not

observed in the J774-C2E2-HAT fusion partner.

I now report that the J774 cell line exhibited a chemiluminescent oxidative burst primed by pre-incubation with a variety of BRMs in a manner analogous to macrophages stimulated in vivo. The 44 h time course of CL illustrated in Fig. 2 attests to the vigor of J774 cells over extended periods of time. The results shown in Figs. 5 and 6 are consistent with the work of Pace and Russel [78] demonstrating that the role of lymphokine such as INF is to sensitize the macrophage to the effects of a second stimulus (LPS) rather than to mediate a full activation. Within this paradigm the ability of bacterial LPS to act as a second stimulus and extend this class of stimulants to include CP is sustained (Fig. 6).

Taniyama and Holden [87] studied the effects of various BRMs on tumor cytotoxicity of macrophage cell lines including J774. They found that LPS at 10 and 100 mg/ml had no effect on the lytic activity of J774 cells. This dosage is more than one thousand-fold greater compared with the dosages used in the present study (Table 1), which are in the low ng/ml range. Ralph and Nakoinz [88] observed growth inhibition as a major effect of high dosages of BCG, LPS and zymosan on J774 cells. Miner et al. [89] found that poly I:C-activated J774 cells were able to lyse B16 melanoma cells. Zahedi and Mortensen [90] recently reported that J774 cells are activated for cytotoxicity by C-reactive protein. The present study extends these results to include LPS and CP as BRMs capable of modulating J774-mediated cytotoxicity. J774 cells may serve as a convenient

model for such diverse macrophage functions as the generation of ROS, the immunosurveillance against malignancy, priming in response to lymphokine for second-signal activating and the in vitro evaluation of immunotherapeutic agents.

CHAPTER 2

MATERIALS AND METHODS

Reagents

Dynorphin A (DYN), ß-endorphin (END), methionine enkephalin (met-ENK), neurotensin, tuftsin, diazepam, γ-aminobutyric acid (GABA), naloxone, norepinephrine (NE), 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol), zymosan A, valinomycin and dimethyl sulfoxide (DMSO) (all from Sigma, St. Louis, MO); 3,3'-dipropylthiadicarbocyanine iodide ($DiSC_3(5)$) (Molecular Probes, Eugene,OR); murine interferon α + ß (Lee Biomolecular Research Inc., San Diego, CA). Recombinant murine γ-interferon (γ-INF) was the generous gift of Genentech, Inc., South San Francisco, CA.

Cells

The murine macrophage cell line J774A.1 was obtained from the American Type Culture Collection (Rockville, MD). This cell line has been described as a reticulum cell sarcoma by Ralph [91-93]. J774 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Grand Island, NY) supplemented with 10% heat inactivated fetal bovine serum (FBS), penicillin (50 U/ml) and streptomycin (250 mg/ml). The viability of cells used in experiments was always >90% as determined by trypan blue exclusion.

Chemiluminescence (CL) assays

The CL response of J774 cells was determined according to a modification of methods reported previously [94,75]. The assay was performed at 37° C in an automated luminometer (Picolite model 6500, Packard Instrument Co., Downers Grove, IL) interfaced with a PC-compatible computer. Luminometry experiments were performed in triplicate. Mixtures of cells, and each specific substance to be tested, were made up in DME/F-12 medium without indicator (Sigma), supplemented with 10% FBS and antibiotics. These suspensions were added to 55 X 12 mm plastic luminometry tubes (Los Alamos Diagnostics, Los Alamos, NM). Each tube contained 2-5 X 10^5 cells in a volume of one ml. Cell suspensions were incubated at 37° C in 5% CO_2 for 18 h prior to the assay to allow the cells to adhere to the bottom of the luminometry tubes. After incubation, 800 l of cell-free medium in each tube were removed and discarded. Repeated examination showed discarded medium contained <0.1% of the original number of cells plated. The CL assay was initiated by the automatic addition of 200 l of a zymosan A suspension containing per ml of complete veronal buffer pH 7.3 [26], 2.5 mg zymosan A, 2 l of a 20 mM solution of luminol in DMSO and 1 g globulin-free bovine serum albumin. The zymosan A suspension was made up in advance and stored frozen at -70 °C in 10 ml aliquots until thawed for use. The photon emission from samples was counted every 4 min for 36 min. The area under curves representing light emission over time was reported as integrated photon emission.

Fluorescent probe studies

The effects of neuroendocrine hormones on the membrane potential of J774 cells were determined according to methods described by Rink et al. [95] for the membrane potential of lymphocytes. Stock solutions of the potential-sensitive fluorescent dye $DiSC_3(5)$ were made up in 95% ethanol at a concentration of 10 M and stored at -20°C. Fluorescence measurements were made with a Perkin-Elmer LS-3 fluorescence spectrophotometer at ambient temperature. Excitation and emission wavelengths were set at 620 nm and 660 nm, respectively. The standard experimental solution (hereafter referred to as buffer) used for measuring the membrane potential contained 139 mM NaCl, 6 mM KCl, 1.25 mM $CaCl_2$, 0.8 mM Mg SO_4, 1 mM Na_2HPO_4, 10 mM Na HEPES, 5.6 mM dextrose; pH 7.2. Stock solutions of valinomycin were made up in 95% ethanol at a concentration of 100 M. Since J774 cells require adherence to a substrate in order to exhibit CL during oxidative burst [75], the effects of neuroendocrine modulators of oxidative burst on membrane potential were studied using adherent populations of cells grown on microscope cover glasses (Fisher, ca. 0.15 mm thick) in DMEM supplemented with 10% heat inactivated FBS and antibiotics. The confluent cover glass cultures were then carefully washed in several volumes of buffer and fitted diagonally into acrylic fluorometry cuvettes (Fisher) containing 2 ml of buffer. Sufficient stock $DiSC_3(5)$ was then added to the cuvette with mild agitation to achieve a final dye concentration of 100 nM and the cuvette was placed in the cell holder and the fluorescence was

monitored at once. Neuroendocrine hormones were dissolved in buffer and added to the cuvettes. Additions of appropriate volumes of buffer or alcohol served as controls for the addition of neuroendocrine hormone or valinomycin, respectively. The effect of mild agitation without any addition had a negligible effect on the fluorescence signal. Data were recorded on a strip chart recorder.

Statistical Analysis

Where appropriate, data were expressed as the mean ± SEM. For multiple group comparisons, the significance of overall differences between the group means was determined by one-way analysis of variance (ANOVA); and between individual groups, by Tukey's test for HSD (honestly significant difference). Significance was defined as $p < 0.05$. Statgraphics software version 2.1 (STSC, Inc., Rockville, MD) was used.

RESULTS

Figure 1 illustrates the CL response of J774 cells incubated with various dosages of γ-INF (0.5-1000 U/ml) for 18 h prior to the assay initiated by the addition of zymosan. Maximal CL was observed at a γ-INF dosage of 5 U/ml. This dosage was used in subsequent experiments where coincubation of a neuroendocrine hormone with γ-INF was desired.

Figures 2a and 2b illustrate the effect of END on the CL response of J774 cells following 18 h of incubation with this opioid peptide at the concentrations indicated, either alone (2a) or with 5 U/ml γ-INF (2b). Maximal CL occurred at an opioid concentration of 10^{-9} M (END alone), or over a broad range (10^{-12}-10^{-8} M) in the case of coincubation of an opioid peptide with γ-INF.

Figure 3a and 3b are similar to Figures 2a and 2b except that dynorphin A (DYN) was substituted for END. The effect of coincubation of γ-INF with DYN was similar to that observed when γ-INF was combined with END, namely the effects were largely additive. Few major alterations in the dose response were observed.

Figure 4 shows the effect of the opiate receptor-antagonist naloxone on DYN and END-enhanced (10^{-9}M each) CL in J774 macrophages. The addition of 10^{-6} M naloxone to otherwise untreated cells had an insignificant effect on CL emission. When this dosage of naloxone was added to cells just prior to their treatment with either opioid peptide, the opioid-induced enhancement of CL was abrogated.

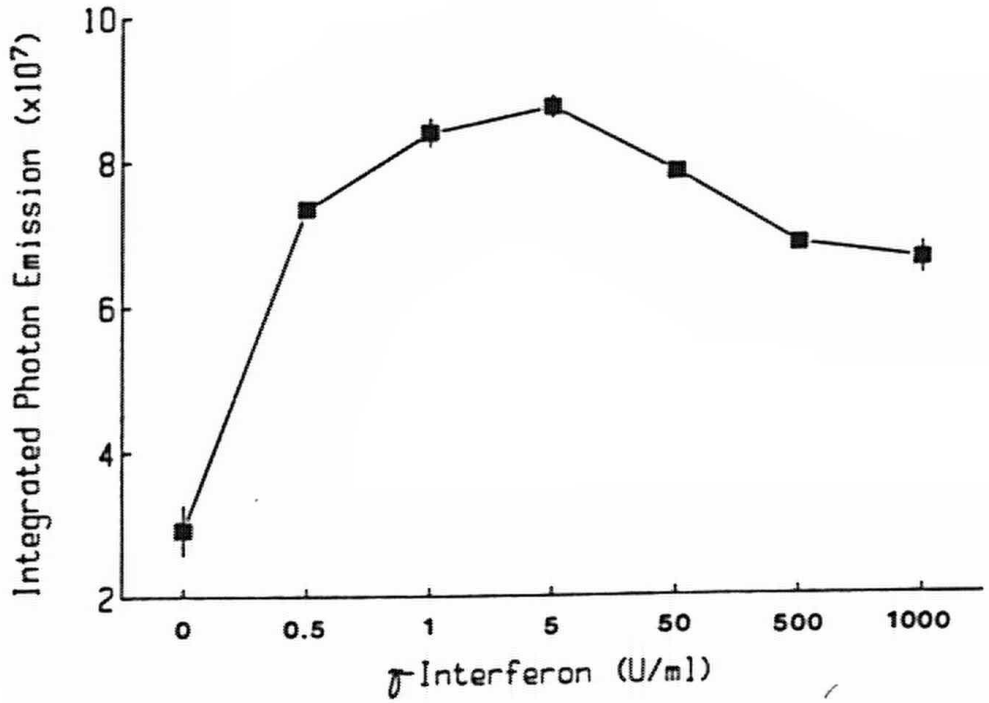

Figure 1. Effect of γ-interferon on chemiluminescence (CL) of J774 cells. Points represent the mean ± SEM (if SEM does not show, it is smaller than the symbol).

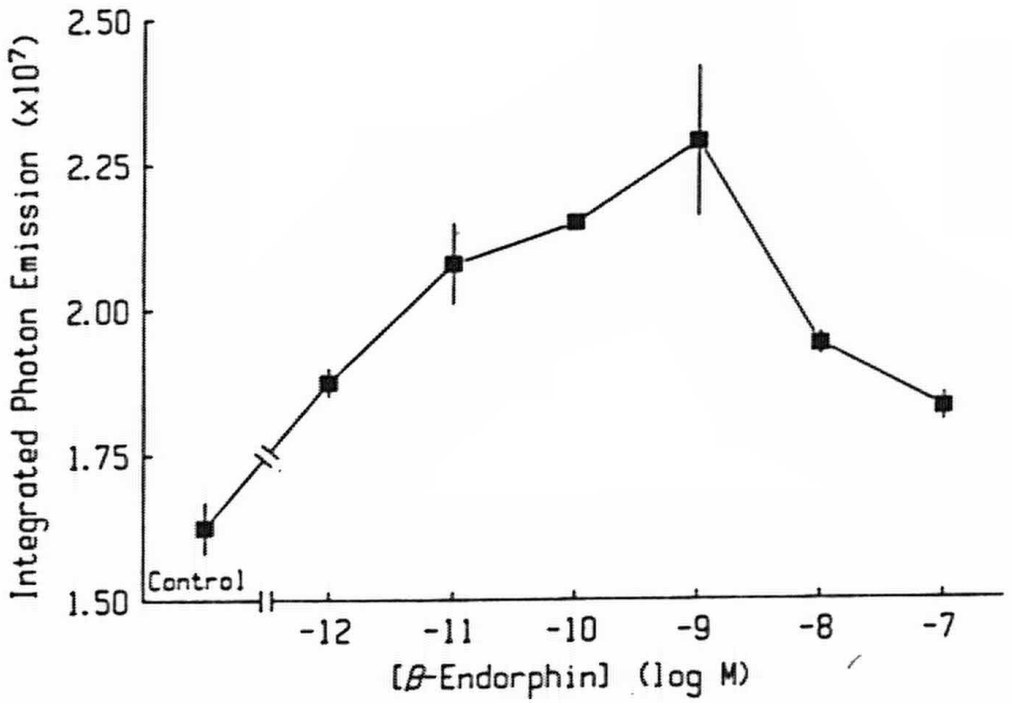

Figure 2a. Effect of ß-endorphin on CL of J774 cells.

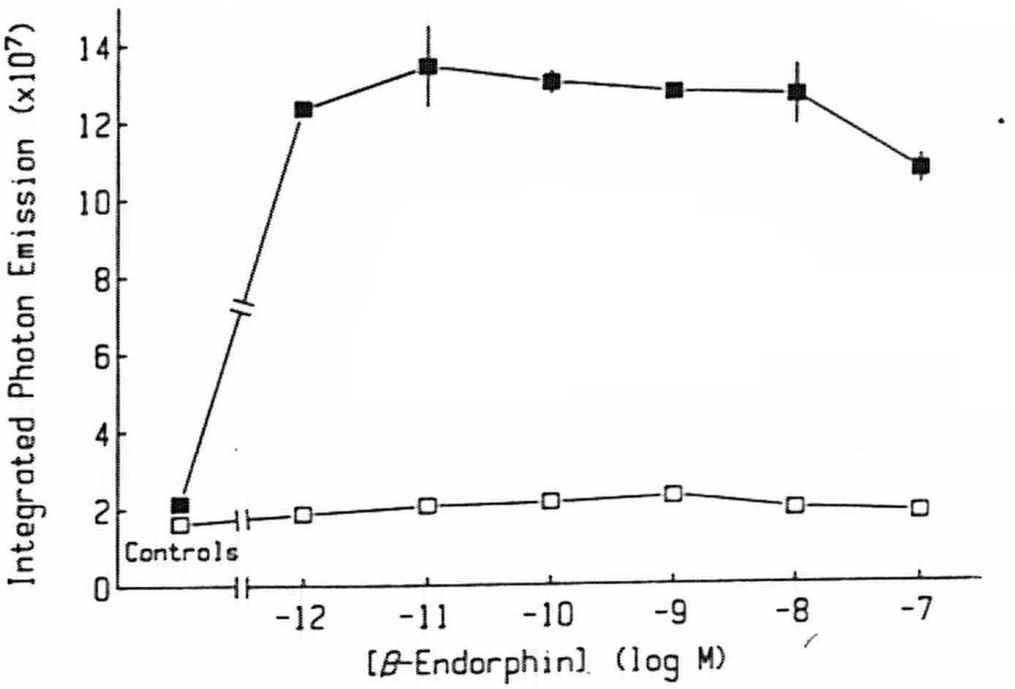

Figure 2b. Effect of ß-endorphin on CL of J774 cells coincubated with 5 U/ml γ-interferon (■——■). Curve from Figure 2a shown for comparison (□——□).

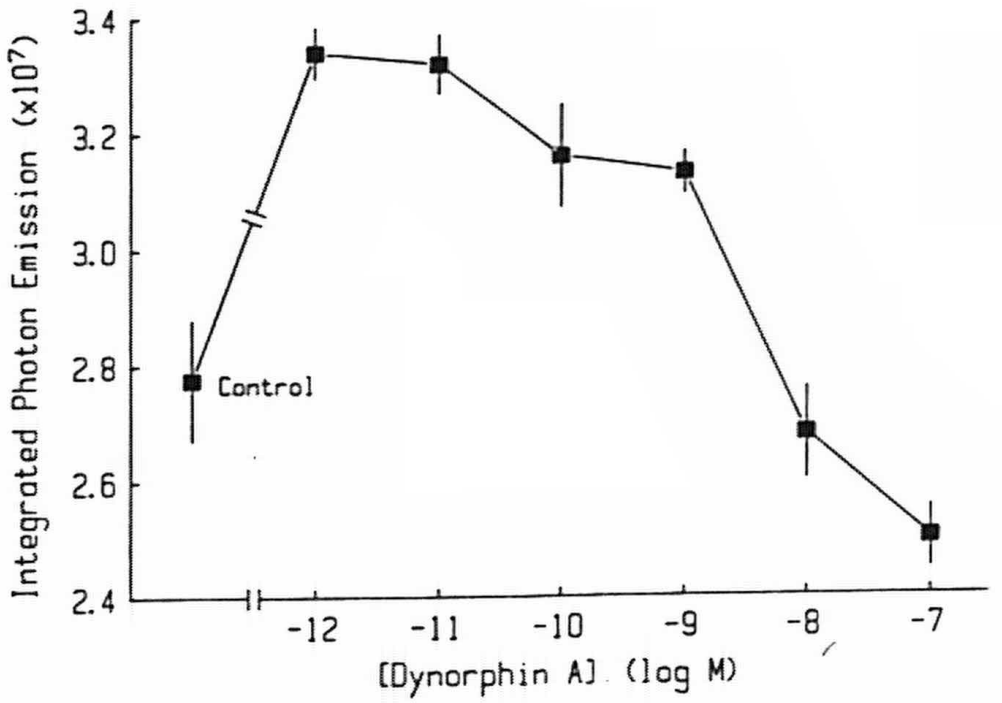

Figure 3a. Effect of dynorphin A on CL of J774 cells.

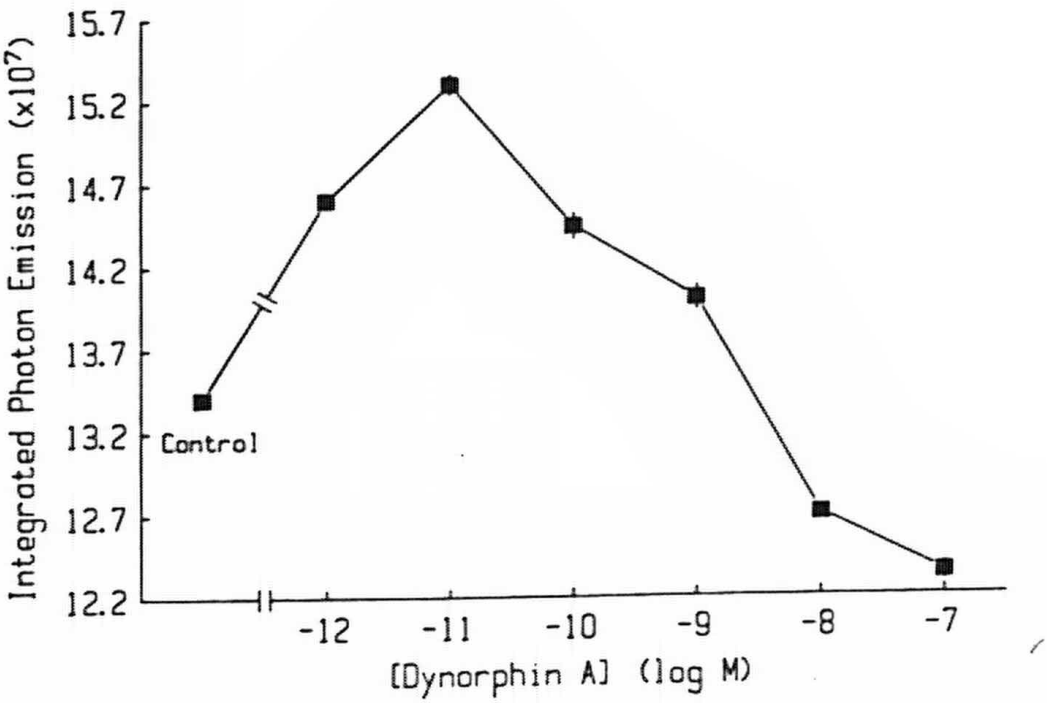

Figure 3b. Effect of dynorphin A on CL of J774 cells coincubated with 5 U/ml γ-interferon.

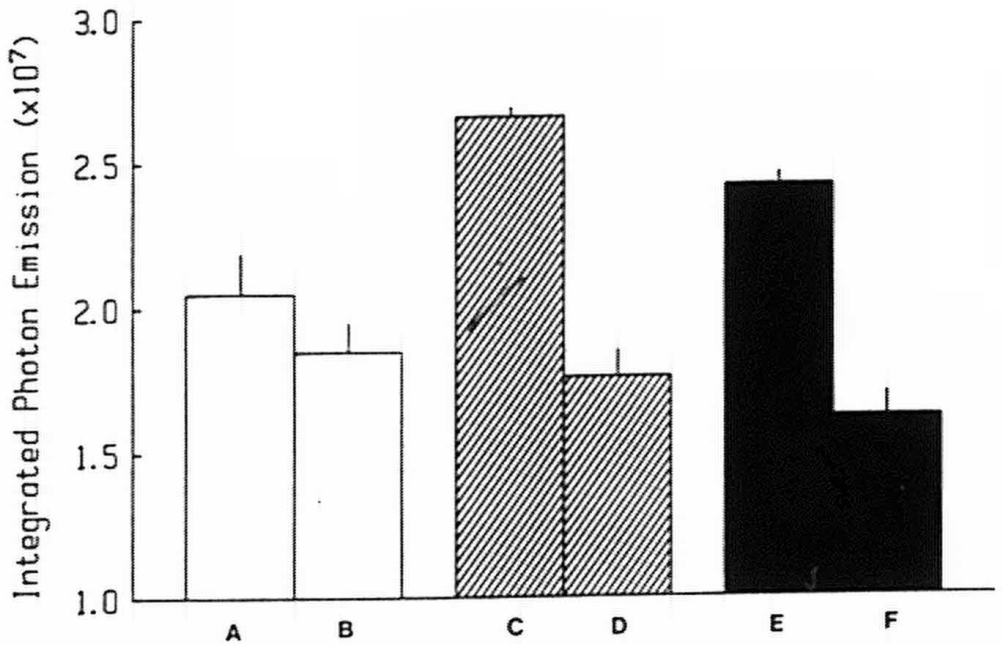

Figure 4. Effect of naloxone (NX) (10^{-4}M) on dynorphin A (DYN) and β-endorphin (END) (10^{-9}M each) -enhanced CL of otherwise untreated J774 cells. A=J774 cells alone, B=cells + NX, C=cells + DYN, D=cells + DYN + NX, E=cells + END, F=cells + END + NX.

When cells were incubated with the dynorphin fragment 1-13 (Figures 5a and 5b), maximal stimulation of CL occurred at the extremely low peptide concentration of 10^{-14}-10^{-12} M, depending only slightly upon the presence or absence of γ-INF (5U/ml). Dynorphin fragment 1-13 was therefore the most potent substance studied here.

In no case did met-ENK enhance J774 CL (Figures 6a and 6b). In fact, a dose-related inhibition of CL was observed which appeared to be enhanced by the addition of γ-INF (Figure 6b).

Figure 7 shows the effect of NT (10^{-11}-10^{-6} M) on CL emission. These effects are characterized by an inverted-U dose response. At a dosage of 10^{-9} M, neurotensin exhibited maximal stimulation of CL. At a concentration of 10^{-6} M, neurotensin significantly inhibited CL. The effects of tuftsin on J774 CL are shown in figures 8a and 8b. Coincubation with γ-INF (Figure 8b) shifted the maximal stimulatory dosage of the peptide from 10^{-6} M (without γ-INF) to 10^{-8} M upon coincubation. The shape of the curve also appeared somewhat subject to the influence of γ-INF, the addition of which was associated with a more symmetrical inverted-U dose response. Figure 9 demonstrates the lack of effect of VIP on the CL emission of J774 cells, a result that was unaltered by coincubation with γ-INF (not shown).

A variety of adrenergic substances have been reported to inhibit macrophage cytotoxicity [96,16], oxidative burst [15] and phagocytosis [14].

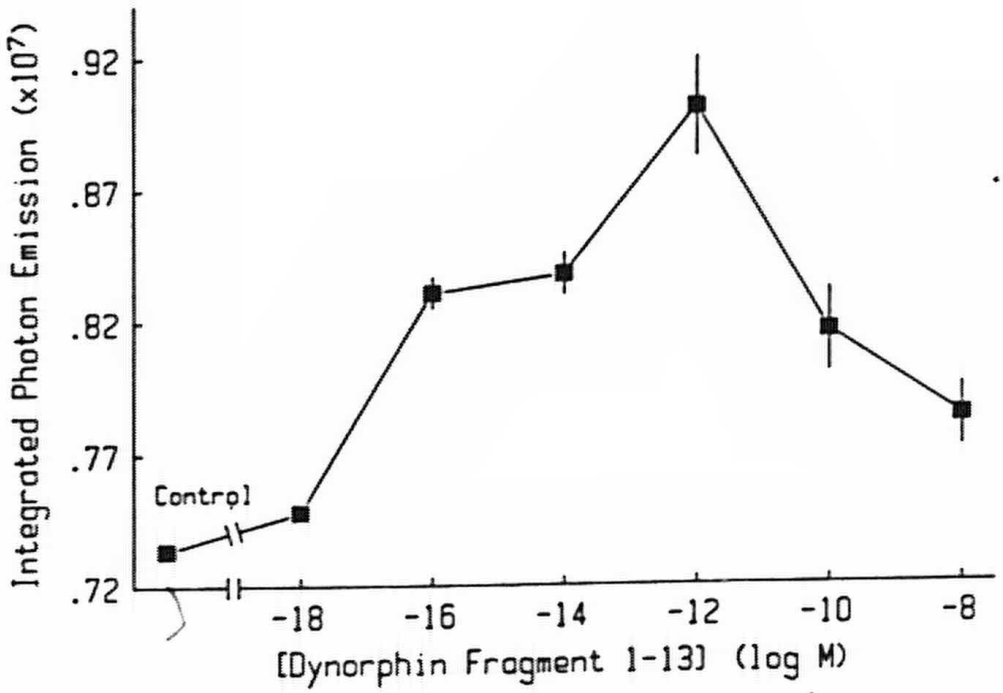

Figure 5a. Effect of dynorphin fragment 1-13 on CL of J774 cells.

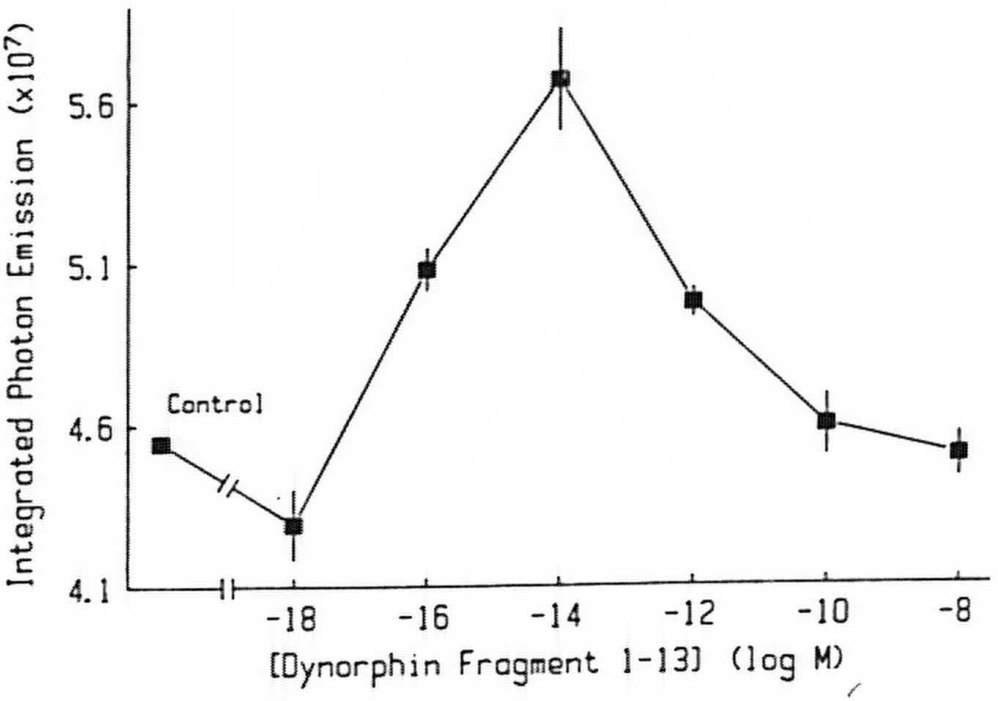

Figure 5b. Effect of dynorphin fragment 1-13 on CL of J774 cells coincubated with 5 U/ml γ-interferon.

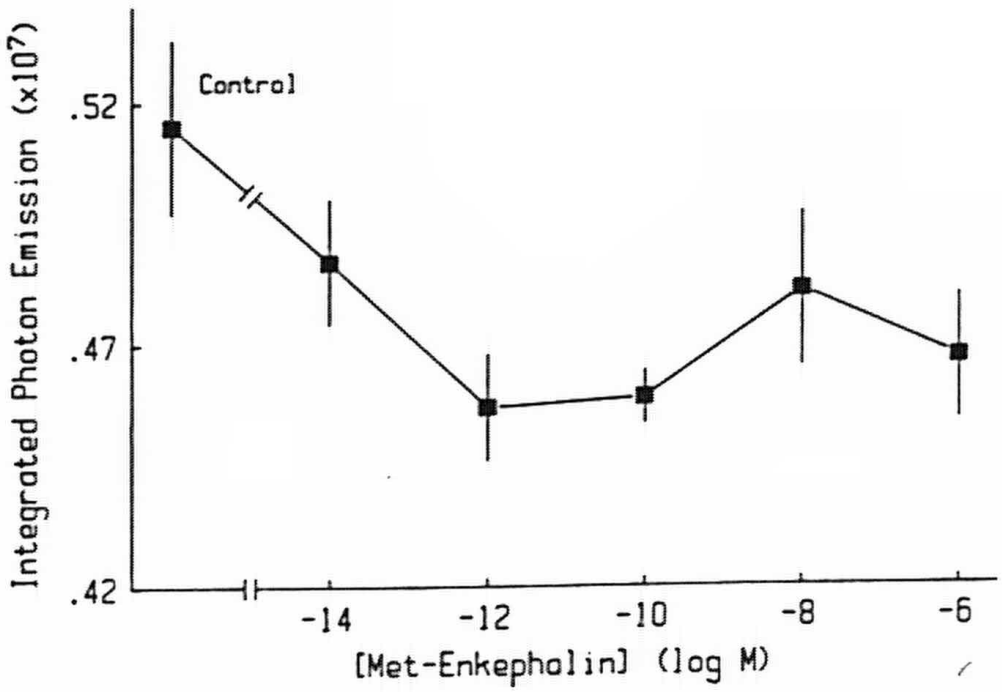

Figure 6a. Effect of met-enkephalin on CL of J774 cells.

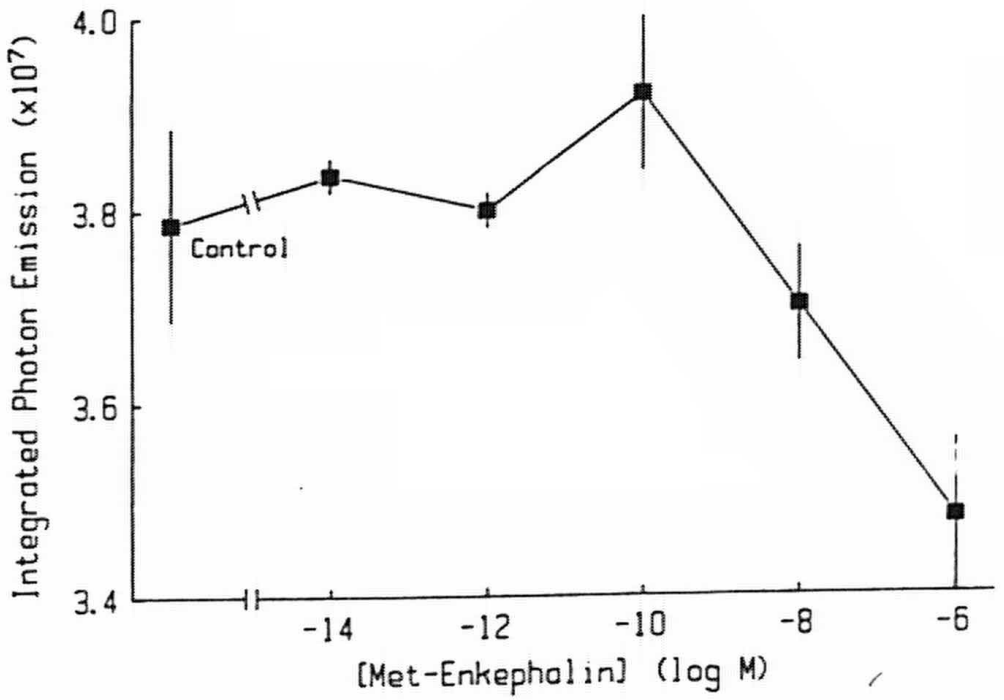

Figure 6b. Effect of met-enkephalin on CL of J774 cells coincubated with 5 U/ml γ-interferon.

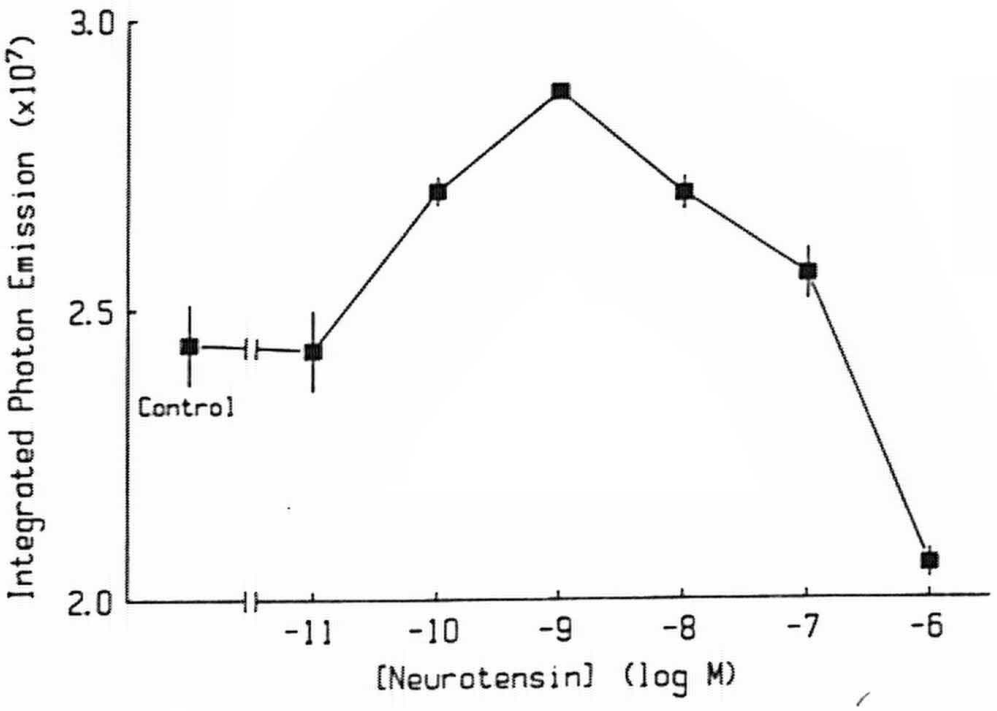

Figure 7. Effect of neurotensin on CL of J774 cells.

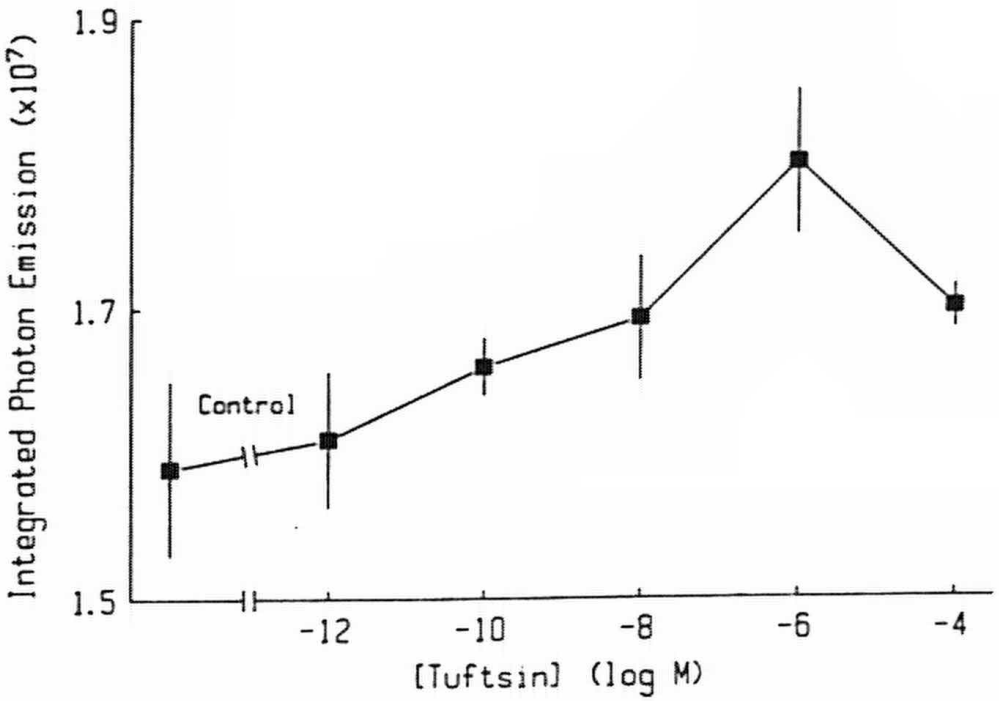

Figure 8a. Effect of tuftsin on CL of J774 cells.

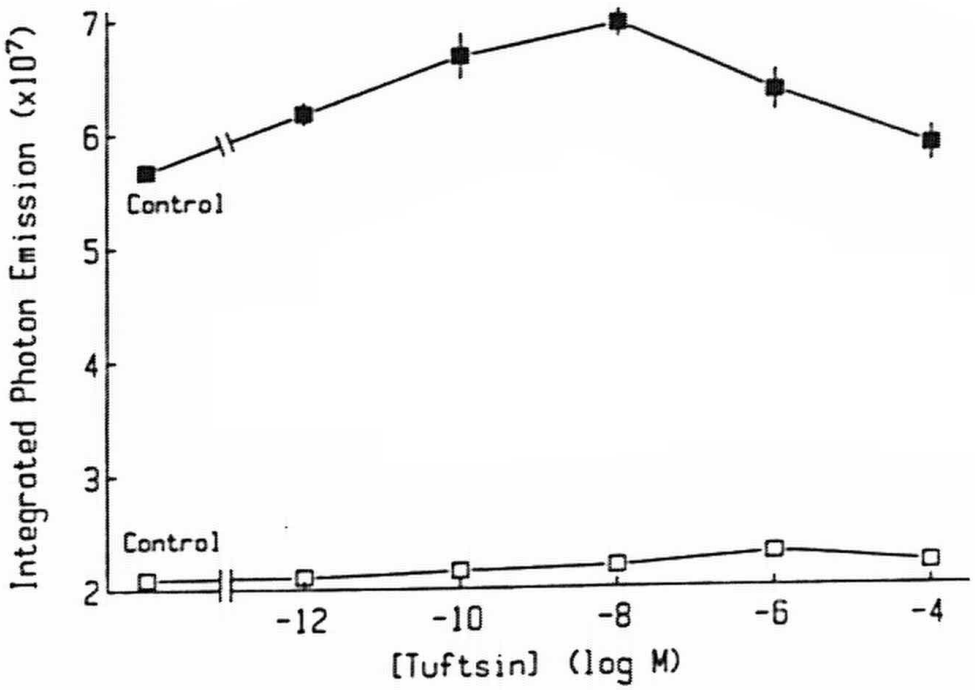

Figure 8b. Effect of tuftsin on CL of J774 cells coincubated with 5 U/ml γ-interferon (■——■). Curve from Figure 8a shown for comparison (○——○).

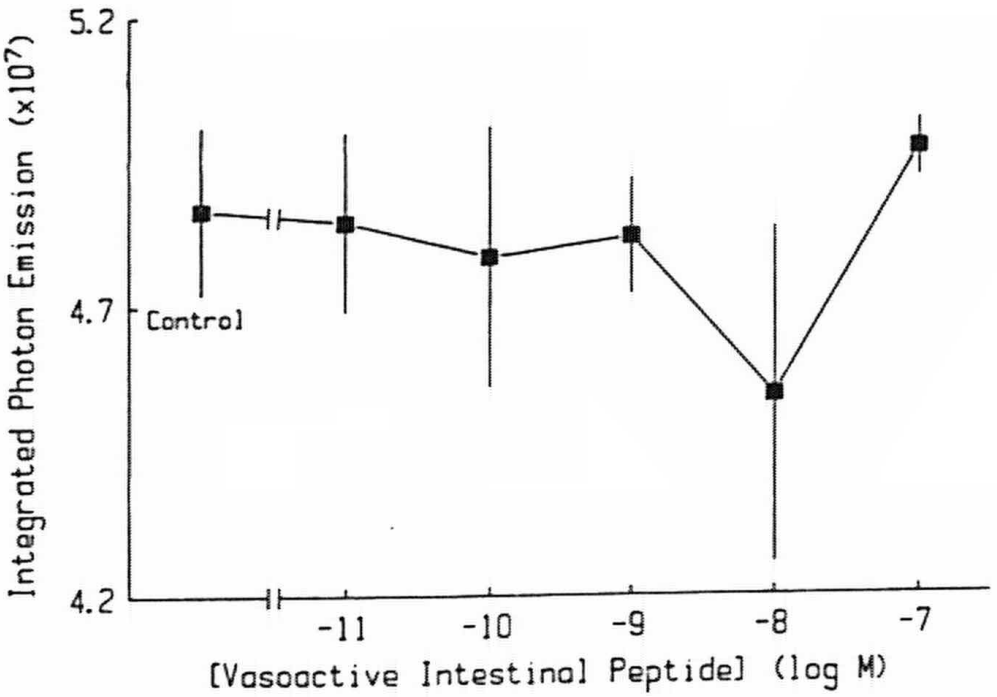

Figure 9. Effect of vasoactive intestinal peptide on CL of J774 cells.

Figure 10a shows that in the absence of added γ-INF there was a biphasic dose response curve for the effects of NE on CL. Dosages over the range of 10^{-11}-10^{-7}M stimulated oxidative burst. Figure 10b indicates an entirely different outcome when cells were incubated with both 5U/ml of γ-INF and NE. In the latter case a dose-related inhibition of CL was observed.

The activity of macrophages has been reported to follow a circadian rhythm [97,98]. Since numerous circadian rhythms are regulated by the pineal gland via its major secretory product, melatonin, the effects of this hormone on J774 CL were studied. Figure 11 shows that the now familiar inverted-U dose response curve is applicable to the effects of melatonin on oxidative burst of J774 macrophages. When cells were coincubated with melatonin and γ-INF, the effects were additive (data not shown).

Lau and associates [94] have recently shown that a water soluble fraction derived from the Chinese medicinal herb *Astralagus membranaceus* possesses potent antitumor activity and exerts a stimulatory effect on J774 CL, inducing a 10-fold increase in photon emission. This herb has been reported to contain the neurotransmitter γ-aminobutyric acid (GABA) [99], a finding which has been confirmed (unpublished observation). Since GABA has been shown to possess macrophage stimulating properties [100] I examined its effects on J774 CL (Figure 12). Dosages above the range shown were associated with a plateau in the level of enhanced CL (not shown).

It is believed that GABA, an inhibitory neurotransmitter, is involved in the

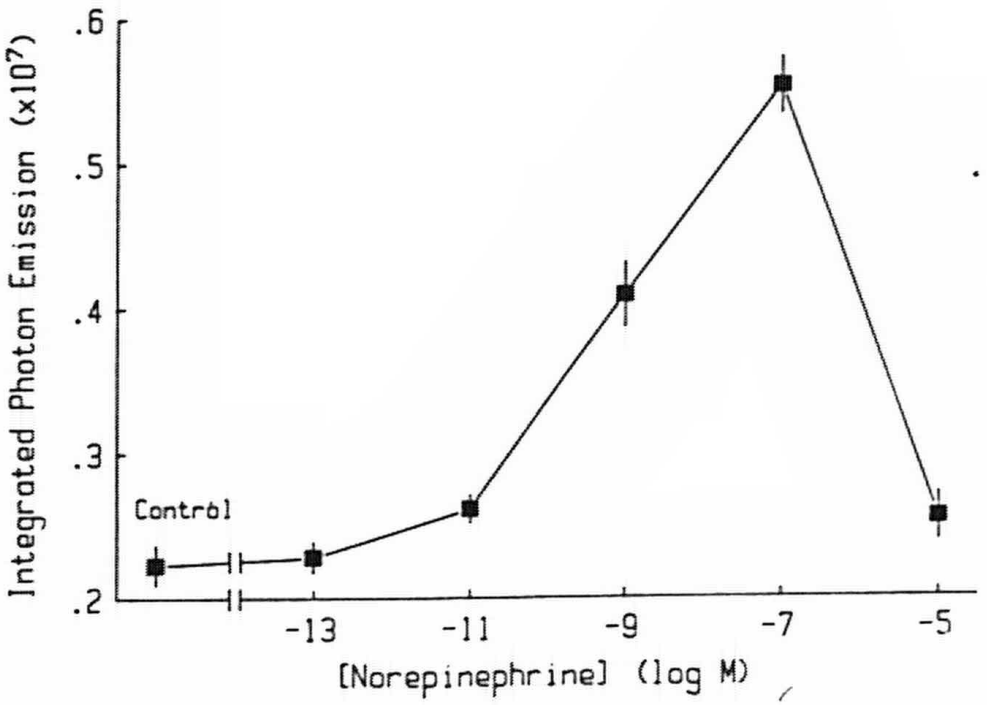

Figure 10a. Effect of norepinephrine on CL of J774 cells.

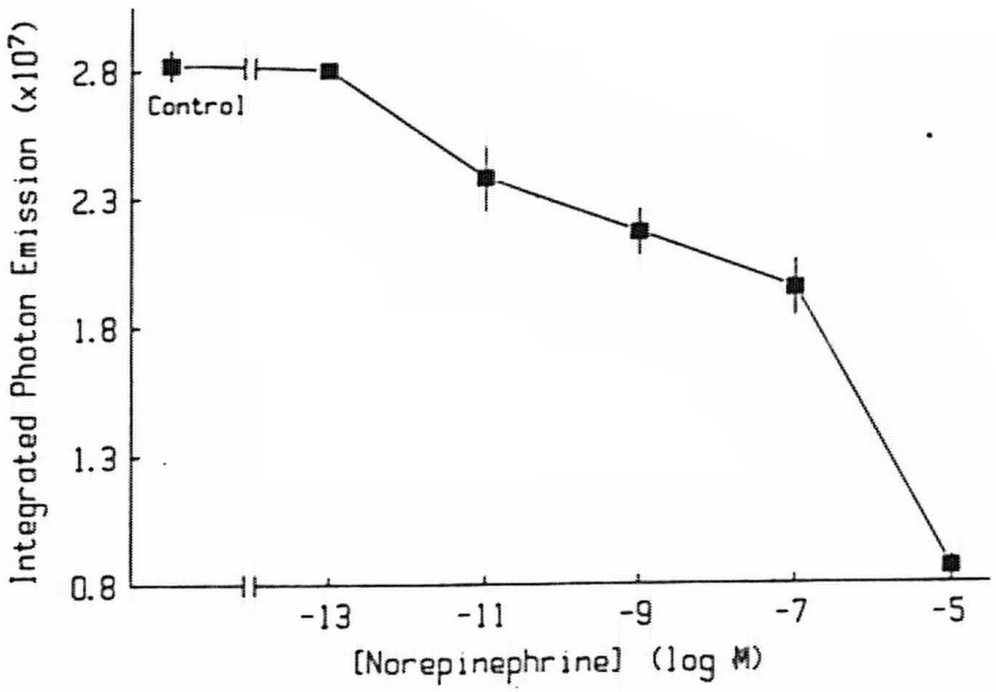

Figure 10b. Effect of norepinephrine on CL of J774 cells coincubated with 5 U/ml γ-interferon.

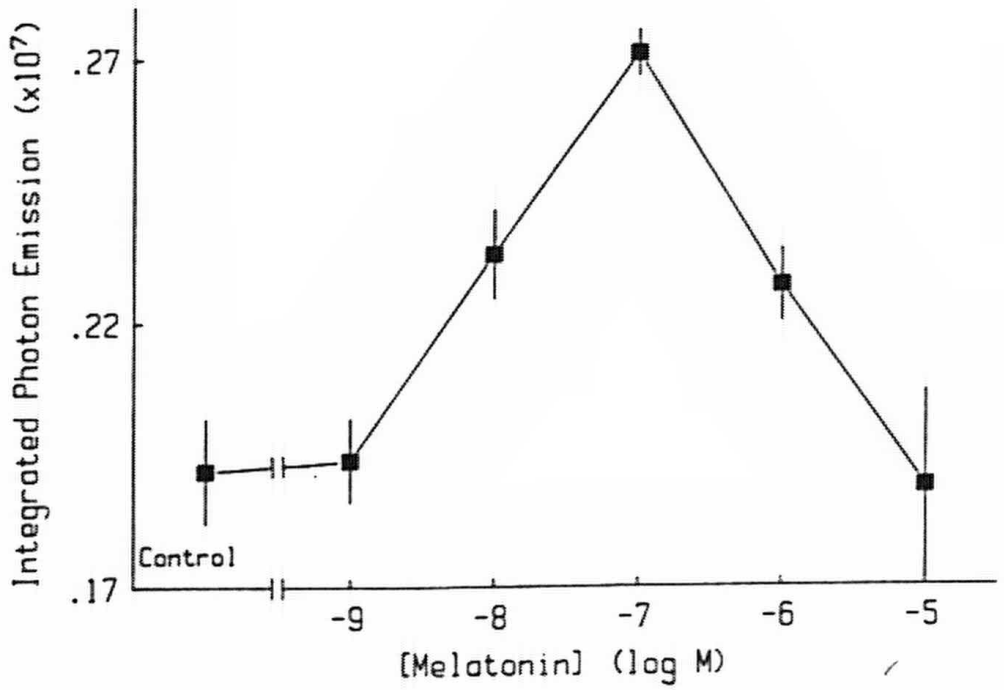

Figure 11. Effect of melatonin on CL of J774 cells.

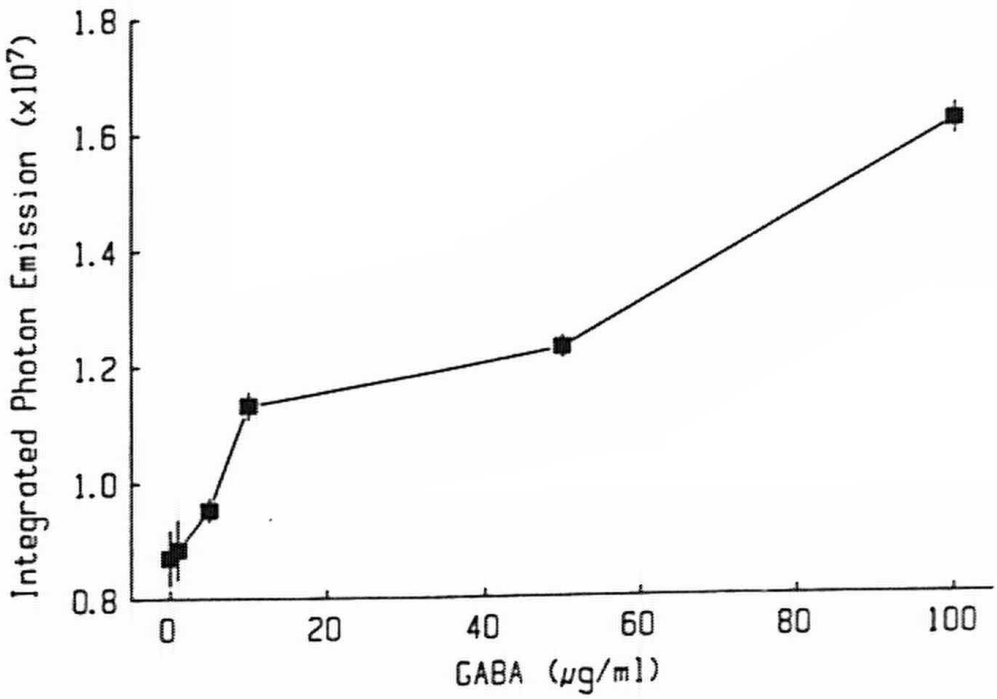

Figure 12. Effect of γ-aminobutyric acid on CL of J774 cells.

mechanism of action of the benzodiazepine class of central depressants. The mechanism involves a three-way complex formed between the benzodiazepine receptor, GABA and the cellular chloride channel [101,102]. Benzodiazepines have therefore become pharmacological probes for studying the physiological role of GABA. The literature reports that the benzodiazepine receptor ligand, diazepam, has a modulatory effect on oxidative burst of macrophages [103]. The effect of diazepam on J774 CL is shown in Figure 13. When γ-INF was added the dose response curve was broadened, and the highest diazepam dosage (10^{-6}M) became supressive ($p < 0.05$).

Effect on membrane potentials

While the mechanism of action by which the diverse neuroendocrine substances reported herein actually modulate the oxidative burst of J774 cells remains obscure, the recent literature suggests that many of these same substances act centrally by altering the membrane potential of specific populations of cells [104]. Using the membrane potential sensitive fluorescent probe $DiSC_3(5)$ I attempted to determine the effects of the neuropeptides and neuroendocrine hormones on the membrane potential of J774 cells. It was only when norepinephrine (NE) was examined that this approach met with success. Figure 14 illustrates the effect of NE (10^{-8}M) on the membrane potential of J774 cells adherent to glass coverslips. NE was dissolved in buffer (see Materials and Methods) and added to the fluorometry cuvette containing the cover glass

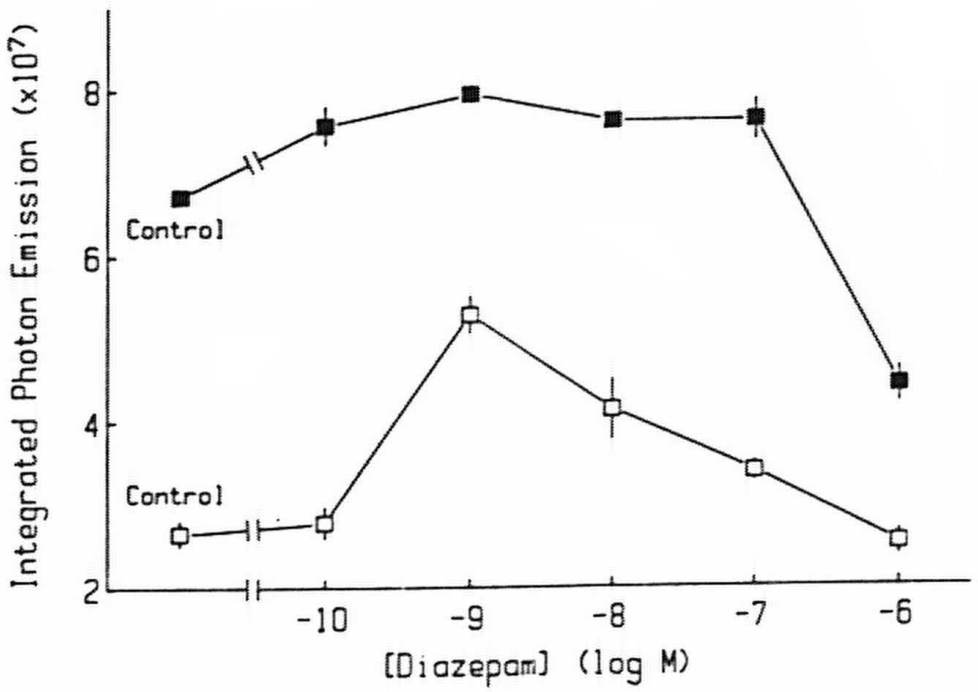

Figure 13. Effect of diazepam on CL of J774 cells (○——○) or on CL of cells coincubated with 5 U/ml of γ-interferon (■——■).

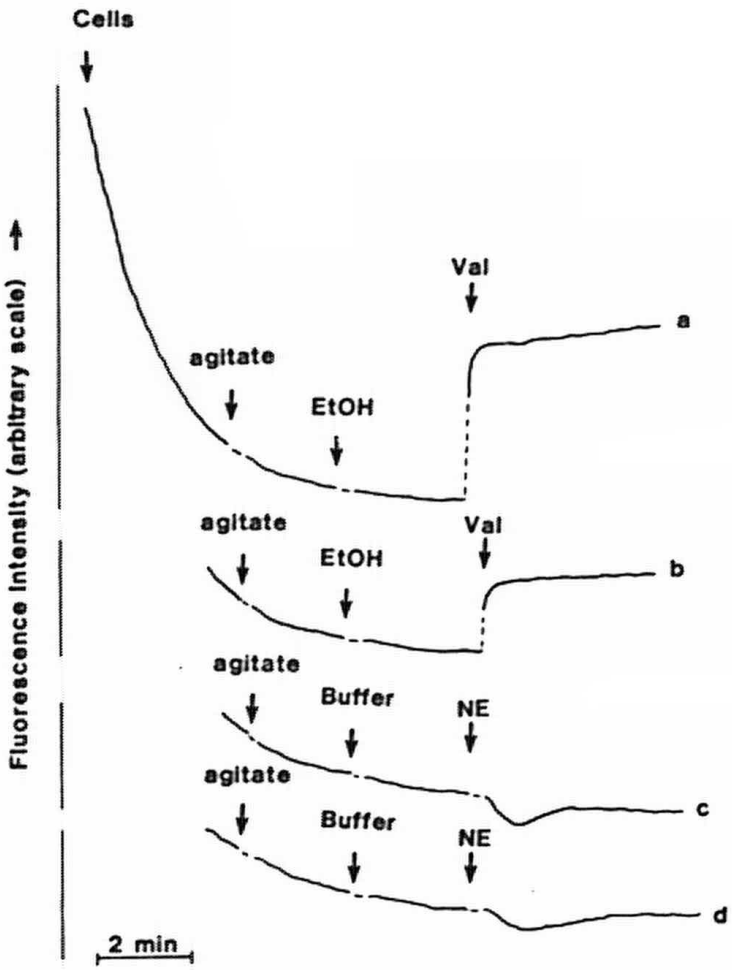

Figure 14. Effect of replicate additions (traces "c" and "d") of 2μl buffer or 2μl buffer solution of norepinephrine (NE) on membrane potential of adherent J774 cells determined by change in fluorescence intensity of adherent J774 cells treated with 100 nm DiSC(3), (final [NE] = 10^{-4}M). Arrows indicate points in time when treatments were added or cuvettes were agitated. Traces "a" and "b" show results of replicate additions (twice each) of 2μl ethanol (EtOH) or 2μl ethanolic valinomycin (VAL) (100μM stock solution). Trace "a" also illustrates decay of fluorescence exhibited by fresh mixtures of cells and dye prepared < 10 sec before examination in fluorometer.

cultures. In these experiments, changes in membrane potential are evidenced by rapid shifts in the fluorescence intensity. The addition of buffer alone served as a negative control. This is illustrated in Figure 14, traces c and d. Upon the addition of buffer alone no change in fluorescence was observed. Similarly, mild agitation had no effect on the fluorescence intensity (Figure 14, traces a-d). Valinomycin depolarizes cells [95] thus inducing a rapid and sustained increase in probe fluorescence intensity (traces a and b). Traces a and b also show that the addition of 2 l of ethanol (EtOH), the solvent for valinomycin, had no effect on fluorescence intensity. Upon the addition of NE such that the final concentration was 10^{-8} M in NE, a rapid transient decrease in fluorescence was observed (Figure 14, traces c and d). This response is consistent with a hyperpolarization.

DISCUSSION

These studies have demonstrated that the chemiluminescent oxidative burst of the J774 macrophage can be modulated to a significant extent by diverse neuropeptides and neuroendocrine hormones. It is of particular importance that in almost every case where modulation of CL was observed, an inverted-U dose response was obtained. A careful examination of the literature indicates that such a response is characteristic of the physiological actions of neuroendocrine substances [105-108]. It is tempting to speculate that the significance of the inverted-U nature of the dose response curves in the cases at hand is that it permits these substances to perform a regulatory function, affording enhanced activity within a certain concentration range, above and below which we find inhibition or reduced stimulation of activity. Although the present investigation did not consider how the family of possible multi-way interactions between the neuroendocrine hormones might contribute to a complex regulatory network, numerous studies have established that such interaction does exist. Catecholamines and opioid peptides exhibit pharmacological interactions in cells with receptors for both classes of ligand [109]. Likewise, melatonin modulates the activity of opioids [110,111]. It remains to be seen whether two or more neuroendocrine hormones which modulate the CL response of J774 cells will exhibit multi-way interactions.

In order to confirm that the opioid peptides END and DYN mediate

their effects (Figures 2 and 3) on CL via classical opiate receptors, the effect of the opiate receptor-antagonist naloxone on opioid peptide-induced modulation of oxidative burst was determined. Indeed naloxone (10^{-6}M) was able to reverse the activity of 10^{-8}M END and DYN (Figure 4). This result is consistent with that of Foster and Moore [20], who determined that DYN-enhanced stimulation of macrophage tumoricidal activity was naloxone-reversible. It is important to characterize the nature of the receptor involved in mediating an opioid-induced response because in some cases such activity is not attributed to an interaction with a true, naloxone-reversible receptor. For example, the effects of opioid peptides on the lymphocytic arm of the immune response has, in some cases, been observed to be mediated by a non-classical opiate receptor [112,113]. The enhancement of natural killer cell cytotoxicity was also enhanced by non-opioid fragments of ß-endorphin [59].

It is interesting to note the exquisite potency with which dynorphin fragment 1-13 enhanced J774 CL (Figure 5a and b). The optimal dosage was on the order of 10^{-14}M (Figures 5a and 5b) with significant activity evident at 10^{-18}-10^{-16}M. This fragment is considered to be an extraordinarily potent opioid peptide [114].

It was surprising to find that methionine enkephalin (met-ENK) was unable to stimulate CL. This opioid peptide is reported to enhance macrophage CL [19], antibody-dependant cytotoxicity [17] and chemotaxis [22]. Peterson et al. [115] indicates that the oxidative burst of human peripheral blood mononuclear

cells is inhibited by END and morphine over a broad range of dosages (10^{-14}-10^{-6}M). This report represents the first study of opioid peptides in a macrophage cell line. The fact that J774 cells are immortal indicates a major difference from macrophages isolated from an intact animal. It is possible that one such difference observed is the general similarity between dose response curves obtained with and without added γ-INF. Foster and Moore [20] report that the addition of a priming stimulus (LPS or α + ß-INF) is required by murine peritoneal macrophages in order to observe any effects of added opioid peptides. Nielson [15] notes that the mechanism of cell activation is critical in determining the outcome of ß-adrenergic modulation of oxidative burst in human PMN, possibly due to differences in calcium mobilization induced by various cell activation pathways.

Neurotensin (NT), tuftsin and vasoactive intestinal peptide (VIP) are three peptide hormones which have previously been shown to modulate macrophage activity [116,16,11,117]. It was therefore of interest to investigate their ability to modulate CL in J774 cells (Figures 7-9).

Most studies of the effects of adrenergic neuroendocrine hormones and drugs on macrophage-monocyte function suggest that suppression of activity is the usual outcome [96,16,118]. I found two distinct patterns, depending on whether or not γ-INF was added: An inverted-U dose response was obtained in the absence of γ-INF (Figure 10a) while dose-related depression of CL was observed when 5U/ml of γ-INF was added (Figure 10b). Upon closer

examination it is interesting that Koff and Dunegan [16] summarize their findings by stating that norepinephrine completely blocks the activation of macrophages by γ-INF. Their figures, however clearly illustrate an inverted-U dose response with NE dosages in the range of 10^{-8}-10^{-9}M significantly enhancing macrophage activation measured by target cytotoxicity. Perhaps there was reluctance to openly confront the prevailing paradigm that substances which increase cAMP levels suppress macrophage activity [119].

Many functions of the macrophage appear to follow a circadian rhythm. Brainard et al. observed that photoperiod length in hamsters affected macrophage count and mononuclear cell hyperplasia [120]. McNulty, Cole and Fox found a circadian rhythm in levels of acid phosphatase in pineal macrophages [121]. Melatonin, the major secretory product of the pineal gland has been shown to modulate cellular immunity in mice [122]. A recent review [111] discussed several effects of melatonin which are relevant in tumor immunology. Against this background it was interesting to find that melatonin modulated CL in J774 cells (Figure 11). The J774 model may find additional application in the study of immunologically relevant interactions between melatonin and opioid peptides and adrenergic substances, as these hormones appear to interact with one another [98].

The inhibitory neurotransmitter γ-aminobutyric acid (GABA) can stimulate macrophage immunity [100] and may account for at least part of the macrophage-stimulating activity we have seen in a Chinese medicinal herb,

Astragalus membranaceus [94]. It was that observation which prompted us to characterize the effects of GABA and its pharmacological probe diazepam, on the CL of J774 cells (Figures 12 and 13). Diazepam and some benzodiazepines are the only pharmacological agents pertinent to the study of psychoneuroimmunology which have been studied in a macrophage cell line, $P338D_1$ [103]. The studies by Lenfant's group [123] represents an experiment in support of the hypothesis that stress-related events have an adverse effect on immunity [54,53,55]. The biphasic dose response obtained for diazepam (Figure 13) is very similar to that found by Zavala and Lenfant [103].

Effect on membrane potential

Ionic channels have been most extensively studied in the nervous system where they function in signal transduction and information processing. Whereas voltage-gated ion channels have been considered unique to cells of the nervous system, it now appears that similar channels are present in most cells of the immune system. Both lymphocytes [124-126] and phagocytes (neutrophils and macrophages) [127-130,104,131,132] possess these attributes, which, on closer examination appear to act at the very interface between the nervous and immune systems. Gallin's elegant review of this subject [133] describes the basic observations which qualify immune cells as possessing classically excitable membranes. Ion channels now appear to play major roles in lymphocyte blastogenesis [134,128,135,136], natural killer cell activity [137], immunoglobulin

function [138] and regulation of oxidative burst [139]. It is well known that cells of the central nervous system (CNS) respond to chemical (neurotransmitter) signals in part by undergoing changes in membrane potential as functions of neurotransmitter exposure. Depolarizations and hyperpolarizations have been recorded in rat hippocampal cells upon local application norepinephrine (NE) [140]. Similarly, cells of the locus coeruleus are hyperpolarized by local application of NE [141].

Because NE hyperpolarizes excitable cells of the CNS, I decided to determine the effects of this catecholamine on the membrane potential of J774 cells, the oxidative burst of which it readily modulated (Figure 14). Fluorescent probes of membrane potential undergo obvious changes in fluorescence intensity as the cells to which they have been added are depolarized or hyperpolarized. In the case of the dye used in the present study, $DiSC_3(5)$, the fluorescence intensity increased as the cells were depolarized and decreased upon hyperpolarization. The effect of depolarization is illustrated by the addition of valinomycin to dyed cells (Figure 14, curves a and b). This substance is a potassium ionophore which causes the cellular potassium to leave the cell under the conditions of the experiment. A concomitant increase in fluorescence appeared upon the addition of valinomycin. The transient decrease in fluorescence upon addition of NE (10^{-8}M final concentration) represents a NE-induced hyperpolarization.

It is hoped that this study will serve to validate and establish the use of the

J774 cell line for investigating the interaction of the macrophage-mediated immune response with components of the neuroendocrine system. The system and methods described may be particularly useful for mechanistic studies, since experiments are free of the many problems associated with the use of primary cultures, whole animals and studies in man. In particular, the J774 macrophage may be helpful in gaining insight into how leukocytes receive and process transmembrane signals of neuroendocrine origin.

REFERENCES

1. Ishigami, T. 1918-1919. The influence of psychic acts on the progress of pulmonary tuberculosis. Am. Rev. Tuberculosis 2:470.

2. Ader, R. 1981. (ed): Psychoneuroimmunology. Academic Press, NY.

3. Palmblad, J. 1981. Stress and immunologic competence: Studies in man. In: Ader, R. (ed): Psychoneuroimmunology. Academic Press, N.Y. p. 229.

4. Monjam, A.A. 1981. Stress and immunologic competence: Studies in animals. In: Ader, R. (ed): Psychoneuroimmunology. Academic Press, N.Y. p. 185.

5. Spengler R.N., Allen R.M., Remick D.G., Strieter, R.M. and Kunkel S.L. 1990. Stimulation of alpha-adrenergic receptor augments the production of macrophage-derived tumor necrosis factor. J. Immunol. 145:1430.

6. Baybutt H.N. and Holsboer, F. 1990. Inhibition of macrophage differentiation and function by cortisol. Endocrinology 127:476.

7. Petty H.R. and Martin S.M. 1989. Combinative ligand-receptor interactions: effects of cAMP, epinephrine, and met-enkephalin on RAW264 macrophage morphology, spreading, adherence, and microfilaments. J. Cell. Physiol. 138:247.

8. Bar-Shavit, Z., Goldman, R., Stabinsky, Y., Gottlieb, P., Fridkin, M., Teichberg, V.I. and Blumberg, S. 1980. Enhancement of phagocytosis - a newly-found activity of substance P residing in its N-terminal tetrapeptide sequence. Biochem. Biophy. Res. Comm. 94,1445.

9. Edwards III, C.K., Ghiasuddin, S.M., Schepper, J.M., Yunger, L.M. and Kelley, K.W. 1988. A newly-defined property of somatotropin: priming of macrophages for production of superoxide anion. Science 239,769.

10. Ignarro, L.J. and George, W.J. 1974. Hormonal control of lysosomal enzyme release from human neutrophils: elevation of cyclic nucleotide levels by autonomic neurohormones. Proc. Nat. Acad. Sci. USA 71,2027.

11. Peck, R. 1987. Neuropeptides modulating macrophage function. Ann. N.Y. Acad. Sci. 496,264.

12. Schultz, R.M., Chirigos, M.A., Stoychkov, J.N., and Pavlidis, N.A. 1979. Factors affecting macrophage cytotoxic activity with particular emphasis on corticosteroids and acute stress. J. Reticuloendothel. Soc. 26,83.

13. Bourne, H.R., Lehrer, R.I., Cline, M.J. and Melmon, K.L. 1971. Cyclic 3',5'-adenosine monophosphate in the human leukocyte: Synthesis, degradation, and effects on neutrophil candidicidal activity. J. Clin. Invest. 50,920.

14. Cox, J.P. and Karnovsky, M.L. 1973. The depression of phagocytosis by exogenous cyclic neuropeptides, prostaglandins, and theophylline. J. Cell. Biol. 59,480.

15. Nielson, C.P. 1987. ß-Adrenergic modulation of the polymorphonuclear leukocyte respiratory burst is dependent upon the mechanism of cell activation. 139,2392.

16. Koff, W.C., and Dunegan, M.A. 1985. Modulation of macrophage-mediated tumoricidal activity by neuropeptides and neurohormones. J. Immunol. 135,350.

17. Foris, G., Medgyesi, G.A. and Hauck, M. 1986. Bidirectional effect of met-enkephalin on macrophage effector functions. Molec. Cell. Biochem. 69,127.

18. Foris, G., Medgyesi, G.A., Nagy, J.T., Varga, Z. 1987. Concentration-dependent effect of met-enkephalin on human polymorphonuclear leukocytes. Ann. N.Y. Acad. Sci. 496,151.

19. Foris, G., Medgyesi, G.A., Gyimesi, E. and Hauck, M. 1984. Met-enkephalin induced alterations of macrophage functions. Molec. Immunol. 21,747.

20. Foster, J.S., and Moore, R.N. 1987. Dynorphin and related opioid peptides enhance tumoricidal activity mediated by murine peritoneal macrophages. J. Leukocyte Biol. 42,171.

21. Simpkins, C.O., Dickey, C.A. and Fink, M.P. 1984. Human neutrophil migration is enhanced by beta-endorphin. Life Sci. 34,2251.

22. van Epps, D.E., and Saland, L. 1984. ß-endorphin and met-enkephalin stimulate human peripheral blood mononuclear cell chemotaxis. J. Immunol. 132,3046.

23. Sharp, B., Keane, W.F., Suh, H.J., Gekker, G., Tsukayama, D., and Peterson, P.K. 1985. Opioid peptides rapidly stimulate superoxide production by human polymorphonuclear leukocytes and macrophages. Endocrinology 117,793.

24. Carr D.J. and Blalock, J.E. 1989. From the neuroendocrinology of lymphocytes toward a molecular basis of the network theory. Horm. Res.31:76.

25. Tosk, J. 1974. Chlorpromazine protection against acridine-induced reversion of a histidine-requiring mutant of *Salmonella typhimurium*. Mutation Res 24:1.

26. Allen, R.C. 1986. Phagocytic leukocyte oxygenation activities and chemiluminescence: a kinetic approach to analysis. In Methods in Enzymology Vol. 133 (DeLuca, M.A.and McElroy, W.D., Eds.) New York:Academic Press, p.449.

27. Allen, R.C. and Loose, L.D. 1976. Phagocytic activation of a luminol-dependent chemiluminescence in rabbit alveolar and peritoneal macrophages. Biochem. Biophys. Res. Comm. 69,245.

28. Allen, R.C., Stjernholm, R.L. and Steele, R.H. 1972. Evidence for the generation of an electronic excitation state(s) in human polymorphonuclear leukocytes and its participation in bacteriocidal activity. Biochem. Biophys. Res. Comm. 47,679.

29. Allen, R.C. and Loose, L.D. 1976. Phagocytic activation of a luminol-dependent chemiluminescence in rabbit alveolar and peritoneal macrophages. Biochem. Biophys. Res. Comm. 69,245.

30. Abrams, S.I. and Brahmi, Z. 1984. Compared mechanisms of tumor cytolysis by human natural killer cells and activated polymorphonuclear leukocytes. J. Immunol. 132,3192.

31. Allen, R.C. 1986. Phagocytic leukocyte oxygenation activities and chemiluminescence: a kinetic approach to analysis. In Methods in Enzymology Vol. 133 (DeLuca, M.A.and McElroy, W.D., Eds.) New York:Academic Press, p.449.

32. Clark, R.A. and Klebanoff, S.J. 1975. Neutrophil-mediated tumor cell cytotoxicity: role of the peroxidase system. J. Exp. Med. 141,1442.

33. Clark, R.A. and Szot, S. 1981. The myeloperoxidase-hydrogen peroxide-halide system as effectors of neutrophil-mediated tumor cell cytotoxicity. J. Immunol. 126,1295.

34. Lichtenstein, A. 1986. Spontaneous tumor cytolysis mediated by inflammatory neutrophils: dependance upon divalent cations and reduced oxygen intermediates. Blood 67,657.

35. Lichtenstein, A. 1987. Stimulation of respiratory burst of murine peritoneal inflammatory neutrophils by conjugation with tumor cells. Cancer Res. 47,2211.

36. Flescher, E., Gonen, P. and Keisari, Y. 1984. Oxidative burst-dependent tumoricidal and tumorostatic activities of paraffin oil-elicited mouse macrophages. JNCI. 72,1341.

37. Murray, H.W. and Cohn, Z.A. 1980. Mononuclear phagocyte antimicrobial and antitumor activity: The role of oxygen intermediates. J. Invest. Dermatol. 74, 285.

38. Nathan, C.F., Silverstein, S.C., Brukner, L.H. and Cohn, Z.A. 1979. Extracellular cytolysis by activated macrophages and granulocytes. II. Hydrogen peroxide as a mediator of cytotoxicity. J. Exp. Med. 149,100.

39. Nathan, C., Brukner, L., Kaplan, G., Unkeless, J. and Cohn, Z.A. 1980. Role of activated macrophages in antibody-dependent lysis of tumor cells. J. Exp. Med. 152,183.

40. Chung, T. and Kim, Y.B. 1988. Two distinct cytolytic mechanisms of macrophages and monocytes activated by phorbol myristate acetate. J. Leukocyte Biol. 44,329.

41. DiStefano, J.F., Beck, G. and Zucker, S. 1983. Mechanism of BCG-activated macrophage-induced tumor cell cytotoxicity: Evidence for both oxygen-dependant and independant mechanisms. Int. Archs. Allergy Appl. Immun. 70,252.

42. Allen, R.C. 1982. Biochemiexcitation: chemiluminescence and the study of biological oxidation reactions. In Chemical and Biological Generation of Excited States (Adam, W. and Cilento, G., Eds.) New York:Academic Press, p. 309.

43. De Baetselier, P. and Schram, E. 1986. Luminescent bioassays based on macrophage cell lines. In Methods in Enzymology Vol. 133 (DeLuca, M.A.and McElroy, W.D., Eds.) New York:Academic Press, p. 507.

44. Hatch, G.E., Gardner, D.E. and Menzel, D.B. 1978. Chemiluminescence of phagocytic cells caused by N-formylmethionyl peptides. J. Exp. Med. 147,182.

45. Zavala, F. and Lenfant, M. 1987. Peripheral benzodiazepines enhance the respiratory burst of macrophage-like P338D_1 cells stimulated by arachidonic acid. Int. J. Immunopharmacol. 9,269.

46. Stendahl, O., Dahlgren, C. and Hed, J. 1982. Physicochemical and functional changes in human leukemic cell line HL-60. J. Cell. Physiol. 112,217.

47. Lane, R.W., Strum, R.J., Borzelleca, J.F. and Carchman, R.A. 1986. Effect of in vitro differentiation on phorbol diester receptor number in human promyelocytic leukemia (HL-60) cells. Cancer Res. 46,3782.

48. Thompson, B.Y., Sivam, G., Britigan, B.E., Rosen, G.M. and Cohen, M.S. 1988. Oxygen metabolism of the HL-60 cell line: Comparison of the effects of monocytoid and neutrophilic differentiation. J. Leukocyte Biol. 43,140.

49. Clement, L.T. and Lehmeyer, J.E. 1983. Regulation of the growth and differentiation of a human monocytic cell line by lymphokines. I. Induction of superoxide anion production and chemiluminescence. J. Immunol. 130,2763.

50. Sheth, B., Dransfield, I., Partridge, L.J. Barker, M.D. and Burton, D.R. 1988. Dibutryl cyclic AMP stimulation of a monocyte-like cell line, U937: a model for monocyte chemotaxis and Fc receptor-related functions. Immunol. 63,483.

51. Sipka, S., Abel, G., Csongor, J., Chihara, G. and Fachet, J. 1985. Effect of lentinan on the chemiluminescence produced by human neutrophils and the murine macrophage cell line C4Mϕ. Int. J. Immunopharmacol. 7,747.

52. Blalock, J.E. 1984. Relationships between neuroendocrine hormones and lymphokines. Lymphokines 9,1.

53. Pert, C.B., Ruff, M.R., Weber, R.J., and Herkenham, M. 1985. Neuropeptides and their receptors: A psychosomatic network. J. Immunol. 135,820s.

54. Hall, R., and Goldstein, A.L. 1981. Neurotransmitters and the immune system. In Psychoneuroimmunology (Ader, R., Ed.) New York: Academic Press, p. 521.

55. Tefcoma, E.S. and Huey, L.Y. 1985. Psychic distress and the immune response. Life Sci. 36,1799.

56. Feldman, R.D., Hunninghake, G.W. and McArdle, W.L.1987. ß-Adrenergic receptor-mediated suppression of interleukin 2 receptors in human lymphocytes. J. Immunol. 139,3355.

57. Gilman, S.C., Schwartz, J.M., Milner, R.J., Bloom, F.E. and Feldman, J.D., 1982. ß-Endorphin enhances lymphocyte proliferative response. Proc. Natl. Acad. Sci. USA 79,226.

58. Ho, W.K.K. and Leung, A. 1979. The effect of morphine addiction on concanavalin A-mediated blastogenesis. Pharmacol. Res. Commun. 11,413.

59. Kay, N., Morley, J.E., and van Ree, J.M. 1987. Enhancement of human lymphocyte natural killing function by non-opioid fragments of ß-endorphin. Life Sci. 40,1083.

60. Hemmick, L.M. and Bidlack, J.M. 1987. ß-Endorphin modulation of mitogen-stimulated calcium uptake by rat thymocytes. Life Sci. 41,1971.

61. Flescher, E., Gonen, P. and Keisari, Y. 1984. Oxidative burst-dependent tumoricidal and tumorostatic activities of paraffin oil-elicited mouse macrophages. JNCI. 72,1341.

62. Celada, A., Gray, P.W., Rinderknecht, E. and Schreiber, R.D. 1984. Evidence for a gamma-interferon receptor that regulates macrophage tumoricidal activity. J. Exp. Med. 160,55.

63. Pace, J.L., Russell, S.W., Schreiber, R.D., Altman, A. and Katz, D.H. 1983. Macrophage activation: priming activity from a T-cell hybridoma is attributable to interferon-gamma. Proc. Natl. Acad. Sci. USA 80,3782.

64. Pace, J.L. and Russell, S.W. 1981. Activation of mouse macrophages for tumor cell killing. I. Quantitative analysis of interactions between lymphokine and lipopolysaccharide. J. Immunol. 126,1863.

65. Pace, J.L., Russell, S.W., LeBlanc, P.A. and Murasko, D.M. 1985. Comparative effects of various classes of mouse interferons on macrophage activation for tumor cell killing. J. Immunol. 134,977.

66. Murray, H.W. and Cohn, Z.A. 1980. Mononuclear phagocyte antimicrobial and antitumor activity: The role of oxygen intermediates. J. Invest. Dermatol. 74, 285.

67. Nathan, C.F., Silverstein, S.C., Brukner, L.H. and Cohn, Z.A. 1979. Extracellular cytolysis by activated macrophages and granulocytes. II. Hydrogen peroxide as a mediator of cytotoxicity. J. Exp. Med. 149,100.

68. Nathan, C., Brukner, L., Kaplan, G., Unkeless, J. and Cohn, Z.A. 1980. Role of activated macrophages in antibody-dependent lysis of tumor cells. J. Exp. Med. 152,183.

69. Allen, R.C., Stjernholm, R.L. and Steele, R.H. 1972. Evidence for the generation of an electronic excitation state(s) in human polymorphonuclear leukocytes and its participation in bacteriocidal activity. Biochem. Biophys. Res. Comm. 47,679.

70. De Baetselier, P. and Schram, E. 1986. Luminescent bioassays based on macrophage cell lines. In Methods in Enzymology Vol. 133 (DeLuca, M.A.and McElroy, W.D., Eds.) New York:Academic Press, p. 507.

71. Ralph, P., Moore, M.A.S. and Nilsson, K. 1976. Lysozyme synthesis by established human and murine histiocytic lymphoma cell lines. J. Exp. Med. 143,1528.

72. Ralph, P. and Nakoinz, I. 1975. Phagocytosis and cytolysis by a macrophage tumor and its cloned cell line. Nature 257,393.

73. Ralph, P., Prichard, J. and Cohn, M. 1975. Reticulum cell sarcoma: an effector cell in antibody-dependent cell-mediated immunity. J. Immunol. 114,898.

74. Pace, J.L., Russell, S.W., LeBlanc, P.A. and Murasko, D.M. 1985. Comparative effects of various classes of mouse interferons on macrophage activation for tumor cell killing. J. Immunol. 134,977.

75. Tosk, J., Lau, B.H.S., Lui, P., Myers, R.C., and Torrey, R.R. 1989. Chemiluminescence in a macrophage cell line modulated by biological response modifiers. J. Leukocyte Biol. 46:103.

76. Adams, D. and Snyderman, R. 1979. Do macrophages destroy nascent tumors? J. Natl. Cancer Inst. 62,1341.

77. Hibbs, J.B., Granger, D.L., Cook, J.L., Lewis, A.M., Clark, W., Hanna, M., Berke, G. and Nathan, C. 1982. Activated macrophage mediated cytotoxicity for transformed target cells. Adv. Exp. Med. Biol. 146,315.

78. Pace, J.L. and Russell, S.W. 1981. Activation of mouse macrophages for tumor cell killing. I. Quantitative analysis of interactions between lymphokine and lipopolysaccharide. J. Immunol. 126,1863.

79. Celada, A., Gray, P.W., Rinderknecht, E. and Schreiber, R.D. 1984. Evidence for a gamma-interferon receptor that regulates macrophage tumoricidal activity. J. Exp. Med. 160,55.

80. Pace, J.L., Russell, S.W., Schreiber, R.D., Altman, A. and Katz, D.H. 1983. Macrophage activation: priming activity from a T-cell hybridoma is attributable to interferon-gamma. Proc. Natl. Acad. Sci. USA 80,3782.

81. Lichtenstein, A., Kahle, J., Berek, J. and Zighelboim, J. 1984. Successful immunotherapy with intraperitoneal *Corynebacterium parvum* in a murine ovarian cancer model is associated with recruitment of tumor lytic neutrophils into the peritoneal cavity. J. Immunol. 133,519.

82. Chedid, L., Carelli, L. and Audibert, F. 1979. Recent developments concerning muramyl dipeptide, a synthetic immunoregulating molecule. J. Reticuloendothel. Soc. 26,631.

83. Doe, W.F. and Henson, P.M. 1978. Macrophage stimulation by bacterial lipopolysaccharide. I. Cytotoxic effect on tumor target cells. J. Immunol. 148,544.

84. Hirose, A., Ikejima, T. and Gill, D.M. 1985. Established macrophage-like cell lines synthesize interleukin-1 in response to toxic shock syndrome toxin. Infect. Immun. 50,765.

85. Sakurai, A., Satomi, N. and Haranaka, K. 1985. Tumour necrosis factor and the lysosomal enzymes of macrophages or macrophage-like cell line. Cancer Immunol. Immunother. 20,6.

86. Damiani, G., Kiyotaki, C., Soeller, W., Sasda, J., Peisach, J. and Bloom, B.R. 1980. Macrophage variants in oxygen metabolism. J. Exp. Med. 152,808.

87. Taniyama, T. and Holden, H.T. 1980. Cytolytic activity against tumor cells by macrophage cell lines and augmentation by macrophage stimulants. Int. J. Cancer 26,61.

88. Ralph, P. and Nakoinz, I. 1977. Direct toxic effects of immunopotentiators on monocytic, myelocytic and histiocytic or macrophage tumor cells in culture. Cancer Res. 37,546.

89. Miner, K.M., Klostergaard, J., Granger, G.A. and Nicolson, G.L. 1983. Differences in cytotoxic effects of activated murine peritoneal macrophages and J774 monocytic cells on metastatic variants of B16 melanoma. JNCI 70,717.

90. Zahedi, K. and Mortensen, R.F. 1986. Macrophage tumoricidal activity induced by human C-reactive protein. Cancer Res. 46,5077.

91. Ralph, P., Moore, M.A.S. and Nilsson, K. 1976. Lysozyme synthesis by established human and murine histiocytic lymphoma cell lines. J. Exp. Med. 143,1528.

92. Ralph, P. and Nakoinz, I. 1975. Phagocytosis and cytolysis by a macrophage tumor and its cloned cell line. Nature 257,393.

93. Ralph, P., Prichard, J. and Cohn, M. 1975. Reticulum cell sarcoma: an effector cell in antibody-dependent cell-mediated immunity. J. Immunol. 114,898.

94. Lau, B.H.S., Ong, P., and Tosk, J. Macrophage chemiluminescence modulated by Chinese medicinal herbs *Astragalus membranaceus* and *Ligustrum lucidum*. Phytotherapy Res., in press.

95. Rink, T.J., Montecucco, C., Hesketh, T.R. and Tsien, R.Y. 1980. Lymphocyte membrane potential assessed with fluorescent probes. Biochim. Biophys. Acta 595,15.

96. Koff, W.C., and Dunegan, M.A. 1986. Neuroendocrine hormones suppress macrophage-mediated lysis of herpes simplex virus-infected cells. J. Immunol. 136,705.

97. Barnes, P.J. 1988. Inflammatory mechanisms and nocturnal asthma. Am. J. Med. 85,64.

98. Maestroni, G.J.M., Conti, A., and Pierpaoli, W. 1987. The pineal gland and the circadian, opiatergic, immunoregulatory role of melatonin. Ann. N.Y. Acad. Sci. 496,67.

99. Hikino, H., Funayama, S. and Endo, K. 1976. Hypotensive principle of *Astragalus* and *Hedysarum* roots. Planta Medica 30,297.

100. Belokrylov, G.A. and Molchanova, I.V. 1987. [Immunostimulating property of GABA]. Biull. Eksp. Biol. Med. 104,345 (Abstract).

101. Bruun-Meyer, S.E. 1987. The GABA/benzodiazepine receptor-chloride ionophore complex: Nature and modulation. Prog. Neuropsychopharmacol. Biol. Psychiatry 11,365.

102. Havoundjian, H., Trullas, R., Paul, S., and Skolnick, P. 1987. A physiological role of the benzodiazepine/GABA receptor-chloride ionophore complex in stress. Adv. Exp. Med. Biol. 221,459.

103. Zavala, F. and Lenfant, M. 1987. Peripheral benzodiazepines enhance the respiratory burst of macrophage-like P338D_1 cells stimulated by arachidonic acid. Int. J. Immunopharmacol. 9,269.

104. Seligmann, B.E. and Gallin, J.I. 1983. Comparison of indirect probes of membrane potential utilized in studies of human neutrophils. J. Cell. Physiol. 115,105.

105. Borrell, J., Kloet, E.R.D. and Bohus, B. 1984. Corticosterone decreases the efficacy of adrenaline to affect passive avoidance retention of adrenalectomized rats. Life Sci. 34,99.

106. Cerro, S.D. and Borrell, J. 1987. ß-Endorphin impairs forced extinction of an inhibitory avoidance response in rats. Life Sci. 41,579.

107. Cicero, J.T., Meyer, E.R. and Bell, R.D. 1988. Characterization and possible opioid modulation of N-methyl-D-aspartic acid induced increases in serum luteinizing hormone levels in the developing male rat. Life Sci. 42,1725.

108. Moon, T.D. 1988. The effect of opiates upon prostatic carcinoma cell growth. Biochem. Biophy. Res. Comm. 153,722.

109. Redgate, E.S., Deupree, J.D. and Axelrod, J. 1986. Interaction of neuropeptides and biogenic amines on cyclic adenosine monophosphate accumulation in hypothalamic nuclei. Brain Res. 365,61.

110. Maestroni, G.J., Conti, A. and Pierpaoli, W. 1987. Role of the pineal gland in immunity: II. Melatonin enhances the antibody response via an opiatergic mechanism. Clin. Exp. Immunol. 68,384.

111. Regelson, W., and Pierpaoli, W. 1987. Melatonin: A rediscovered antitumor hormone? Its relation to surface receptors; sex steroid metabolism, immunologic response, and chronobiologic factors in tumor growth and therapy. Cancer Invest. 5,379.

112. Hazum, E., Chang, K.J. and Cuatrecasas, P. 1979. Specific nonopiate receptors for ß-endorphin. Science 205,1033.

113. McCain, H.W., Lamster, I.B., Bozzone, J.M. and Grbic, J.T. 1982. ß-Endorphin modulates human immune activity via non-opiate receptor mechanisms. Life Sci. 31,1619.

114. Goldstein, A., Tachibana, S., Lowney, L.I., Hunkapiller, M. and Hood, L. 1979. Dynorphin-(1-13), and extraordinarily potent opioid peptide. Proc. Natl. Acad. Sci. USA 76,6666.

115. Peterson, P.K., Sharp, B., Gekker, G., Brummitt, C., and Keane, W.F. 1987. Opioid-mediated suppression of cultured peripheral blood mononuclear cell respiratory burst activity. J. Immunol. 138,3907.

116. Goldman, R., and Bar-Shavit, Z. 1983. On the mechanism of the augmentation of the phagocytic capability of phagocytic cells by tuftsin, substance P, neurotensin, and kentsin and the interrelationship between their receptors. Ann. N.Y. Acad. Sci. 419,143.

117. Stabinsky, Y., Bar-Shavit, Z., Fridkin, M, and Goldman, R. 1980. On the mechanism of action of the phagocytosis-stimulating peptide tuftsin. Molec. Cell. Biochem. 30,71.

118. Schultz, R.M., Pavlidis, N.A., Stoychkov, J.N. and Chirigos, M.A. 1979. Prevention of macrophage tumoricidal activity by agents known to increase cellular cyclic AMP. Cell. Immunol. 42,71.

119. Koopman, W.J., Gillis, M.H. and David, J.R. 1973. Prevention of MIF activity by agents known to increase cellular cyclic AMP. J. Immunol. 110,1609.

120. Brainard, G.C., Knobler, R.L., Podolin, P.L., Lavasa, M., and Lublin, F.D. 1987. Neuroimmunology: Modulation of hamster immune system by photoperiod. Life Sci. 40,1319.

121. McNulty, J.A., Cole, A.A., and Fox, L.M. 1988. Day-night variability in acid phosphatase activity of pineal gland macrophages. Neurosci. Lett. 90,254.

122. Maestroni, G.J.M., Conti, A., and Pierpaoli, W. 1986. Role of the pineal gland in immunity: Circadian synthesis and release of melatonin modulates the antibody response and antagonizes the immunosuppressive effect of corticosterone. J. Neuroimmunol. 13,19.

123. Zavala, F., and Lenfant, M. 1987. Benzodiazepines and PK 11195 exert immunomodulating activities by binding on a specific receptor on macrophages. Ann. N.Y. Acad. Sci. 496,240.

124. Matteson, D.R., Deutsch, C. 1984. K channels in T lymphocytes: a patch clamp study using monoclonal antibody adhesion. Nature 307,468.

125. Wilson, H.A. and Chused, T.M. 1985. Lymphocyte membrane potential and Ca^{2+}-sensitive potassium channels described by oxonol dye fluorescence measurements. J. Cell. Physiol. 125,72.

126. Witkowski, J. and Micklem, H.S. 1985. Decreased membrane potential of T lymphocytes in ageing mice: flow cytometric studies with a carbocyanine dye. Immunology 56,307.

127. Bashford, C.L. and Pasternak, C.A. 1985. Plasma membrane potential of neutrophils generated by the Na^+ pump. Biochim. Biophy. Acta. 817,174.

128. Jenssen, H.L., Redmann, K., and Mix, E. 1986. Flow cytometric estimation of transmembrane potential of macrophages--A comparison with microelectrode measurements. Cytometry 7,339.

129. Korchak, H.M., and Weissmann, G. 1978. Changes in membrane potential of human granulocytes antecede the metabolic responses to surface stimulation. Proc. Natl. Acad. Sci. USA 75,3818.

130. Randriamampita, C., and Trautmann, A. 1987. Ionic channels in murine macrophages. J. Cell Biol. 105,761.

131. Seligmann, B.E., Gallin, E.K., Martin, D.L., Shain, W. and Gallin, J.I. 1980. Interaction of chemotactic factors with human polymorphonuclear leukocytes: studies using a membrane potential-sensitive cyanine dye. J. Membrane Biol. 52,257.

132. Tatham, P.E.R., Delves, P.J., Shen, L. and Roitt, I.M. 1980. Chemotactic factor-induced membrane potential changes in rabbit neutrophils monitored by the fluorescent dye 3,3'-dipropylthiadicarbocyanine iodide. Biochim. Biophys. Acta 602,285.

133. Gallin, E.K. 1986. Ionic channels in leukocytes. J. Leuk. Biol. 39,241.

134. DeCoursey, T.E., Chady, K.G., Gupta, S. and Cahalan, M.D. 1984. Voltage-gated K^+ channels in human T lymphocytes: a role in mitogenesis? Nature 307,465.

135. Kiefer, H., Blume, A.J. and Kaback, H.R. 1980. Membrane potential changes during mitogenic stimulation of mouse spleen lymphocytes. Proc. Natl. Acad. Sci. USA 77,2204.

136. Tatham, P.E.R. and Delves, P.J. 1984. Flow cytometric detection of membrane potential changes in murine lymphocytes induced by concanavalin A. Biochem. J. 221,137.

137. Schlichter, L., Sidell, N., and Hagiwara, S. 1986. Potassium channels mediate killing by human natural killer cells. Proc. Natl. Acad. Sci. USA 83,451.

138. Young, J.D., Unkeless, J.C., Kaback, H.R., and Cohn, Z.A. 1983. Mouse macrophage Fc receptor for IgGγ2b/γ1 in artificial and plasma membrane vesicles functions as a ligand-dependent ionophore. Proc. Natl. Acad. Sci. USA 80,1636.

139. Wright, J., Maridonneau-Parini, I., Cragoe Jr., E.J., Schwartz, J.H. and Tauber, A.I. 1988. The role of the Na^+/H^+antiporter in human neutrophil NADPH-oxidase activation. J. Leuk. Biol. 43,183.

140. Madison, D.V. and Nicoll, R.A. 1986. Actions of noradrenaline recorded intracellularly in rat hippocampal CA1 pyramidal neurones, in vitro. J. Physiol. 372,221.

141. Aghajanian, G.K. and Wang, Y.Y. 1987. Common alpha 2- and opiate effector mechanisms in the locus coeruleus: intracellular studies in brain slices. Neuropharmacol. 26,796.

Abstract

MODULATION OF MACROPHAGE CHEMILUMINESCENCE BY BIOLOGICAL RESPONSE MODIFIERS AND NEUROENDOCRINE HORMONES

by

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Neuroendocrine mediators have been shown to influence a broad spectrum of immune functions suggesting the existence of a neuroimmune regulatory axis effecting feedback control of the immune response. Most studies in support of this paradigm have employed either intact animals or immune cells derived from blood and other tissues. Under these conditions it becomes difficult to cope with numerous variables associated with the complex milieu of the immune system and the interpretation of data. An in vitro model using a murine macrophage cell line, J774, was developed to circumvent these problems. Using this model, macrophages activated by exposure to the biological response modifiers lipopolysaccharide, bacillus Calmette-Guerin, *Corynebacterium parvum*, $\alpha + \beta$ interferon and γ-interferon exhibited a readily observed and quantitated chemiluminescent oxidative burst. This activity was modulated by the neuropeptides ß-endorphin, dynorphin A, neurotensin and tuftsin as well as the neuroendocrine hormones norepinephrine, γ-aminobutyric acid and the pineal

hormone melatonin. In the case of norepinephrine, the mechanism of action may involve hormone-mediated hyperpolarization of the macrophage membrane potential.