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Basal nitric oxide limits immune, nervous and cardiovascular excitation: human endothelia express a mu opiate receptor

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Abstract

Nitric oxide (NO) is a major signaling molecule in the immune, cardiovascular and nervous systems. The synthesizing enzyme, nitric oxide synthase (NOS) occurs in three forms: endothelial (e), neuronal (n) and inducible (i) NOS. The first two are constitutively expressed. We surmise that in many tissues there is a basal level of NO and that the actions of several signaling molecules initiate increases in cNOS-derived NO to enhance momentary basal levels that exerts inhibitory cellular actions, via cellular conformational changes. It is our contention that much of the literature concerning the actions of NO really deal with i-NOS-derived NO. We make the case that cNOS is responsible for a basal or 'tonal' level of NO; that this NO keeps particular types of cells in a state of inhibition and that activation of these cells occurs through disinhibition. Furthermore, naturally occurring signaling molecules such as morphine, anandamide, interleukin-10 and 17-β-estradiol appear to exert, in part, their beneficial physiological actions, i.e., immune and endothelial down regulation by the stimulation of cNOS. In regard to opiates, we demonstrate the presence of a human endothelial mu opiate receptor by RT-PCR and sequence determination, further substantiating the role of opiates in vascular coupling to NO release. Taken together, cNOS derived NO enhances basal NO actions, i.e., cellular activation state, and these actions are further enhanced by iNOS derived NO. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Nitric oxide; Morphine; Endothelial mu opiate receptor; Estrogen; Anandamide; Opiate receptor

Contents

Abbreviations: ACH, acetylcholine; AIDS, acquired immune deficiency syndrome; E_2-BSA (CALLA), acute lymphoblastic leukemic antigen; 17β-estradiol conjugated to bovine serum albumin; ACTH, adrenocorticotropin; CNS, central nervous system; GABA, gamma amino buteric acid; GnRH, gonadotropin releasing hormone; IL, interleukin; IFN-γ, interferon-γ; ITA, internal thoracic artery; LPS, lipopolysaccharide; L-NAME, N^G-nitro-L-arginine methyl ester; NO, nitric oxide; NOS, nitric oxide synthase; (e) endothelial, (n) neuronal; (c) constitutive and (i) inducible NOS; NK-kB, nuclear factor-kappa B; SNAP, S-nitroso-*N*-acetyl-DL-penicillamine; THC, tetrahydrocannabinol; TNF, tumor necrosis factor.

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1. Introduction

Nitric oxide (NO) is a major signaling molecule in several systems including the immune, cardiovascular and nervous systems (Cooke and Dzau, 1997; Faraci and Heistad, 1998; Kinoshita et al., 1997; Moncada et al., 1988, 1991; Stefano et al., 1996a). Perhaps as importantly, because it is a gas, NO produced at one site can have an effect on tissues at a distance (Deutsch et al., 1997; Prevot et al., 1999).

NO is produced from L-arginine by the enzyme NO synthase (NOS) (Moncada and Higgs, 1993; Moncada et al., 1991). NOS occurs in three forms: endothelial (e), neuronal (n) and inducible (i) NOS. The first two are constitutively expressed and Ca^{2+} dependent whereas inducible (i) NOS is Ca^{2+} independent. The three forms of NOS are encoded for on three distinct genes on chromosomes 7, 12 and 17, respectively (Cooke and Dzau, 1997; Faraci and Heistad, 1998; Kinoshita et al., 1997; Moncada, 1997). In general, n- and e-NOS depend on intracellular calcium transients and release NO in the nM range whereas iNOS, following an induction/latency period, can release NO in the μM range for extended periods of time (Cooke and Dzau, 1997; Faraci and Heistad, 1998; Fimiani et al., 1999a,b; Kinoshita et al., 1997; Moncada et al., 1988; Prevot et al., 1999a; Stefano et al., 1996a, 1998a). The presence of constitutive and inducible forms of NOS suggests that they may have distinct functions. It is our contention that much of the literature concerning the actions of NO really deal with i-NOS-derived NO. We will make the case that cNOS is responsible for a basal or 'tonal' level of NO; that this NO keeps particular types of cells in a state of inhibition and that activation of these cells occurs through disinhibition.

2. Constitutive nitric oxide (NO)

c- and i-NOS can be distinguished on the basis of the length of time necessary to see an increase in levels of NO and the length of time these elevated levels can be maintained. NO derived from cNOS may occur in two functional forms: the first is always present at low 'tonal' or 'basal' levels; this basal level can be slightly increased for a short time in response to certain signals, e.g., acetylcholine (ACH; see (Moncada et al., 1988)). This brief enhanced release of cNOS derived NO can have profound physiological actions, which are evident long after NO has returned to its basal level, for a longer period of time (Magazine et al., 1996). For example, endothelial cells briefly exposed to morphine and e-NOS change their shape from elongated to round, a process that takes several hours (Magazine et al., 1996).

iNOS is induced by various signal molecules, e.g., proinflammatory cytokines (Moncada et al., 1991; Radomski et al., 1990; Stefano et al., 1998a). The induction of iNOS is usually seen after a 3–4 h delay; iNOS is capable of producing NO for 24–48 h (Stefano et al., 1998a; Radomski et al., 1990). These data suggest first, that NO is always present and, second, that the levels of NO can be regulated either rapidly or slowly depending on the organism's needs. The presence of different regulatory processes imply that NO has different functions, that the levels of NO must be progressively increased in order for it to exert its function, or some combination of the two.

NO functions as a vascular, immune and neural signal molecule and also has general antibacterial, antiviral actions and the ability to down-regulate proinflammatory events (Kubes and Granger, 1992; Kubes et al., 1991, 1993; Kurose et al., 1993; Niu et al., 1995; Stefano, 1998; Stefano et al., 1998a,b,c). In this review, we will pay particular attention to the activities of cNOS derived NO. We hypothesize that certain classes of cells are always 'on', i.e., respond to environmental changes, and that this low level of NO provides an organism with a major pathway that functions to dampen microenvironmental 'noise' which would otherwise nonspecifically and inappropriately activate them. NO may control the threshold for activation of these cells. This kind of activation really represents a disinhibition process, i.e., an overcoming of the inhibitory influence of NO by changing the balance between basal NO and the levels of excitatory signals (Fig. 1). For example, when excitatory signals, i.e., interleukin-1, are present at detectable levels the tonal inhibition due to NO is overcome and activation occurs. In this review we will critically examine this hypothesis by analyzing the role(s) of NO in the immune, nervous and cardiovascular systems. We will pay particular attention to the evidence for differential activity of c- and i-NOS and determine if they act via common processes. Lastly, we will speculate as to a possible functional rationale for cNOS derived NO tonal inhibition.

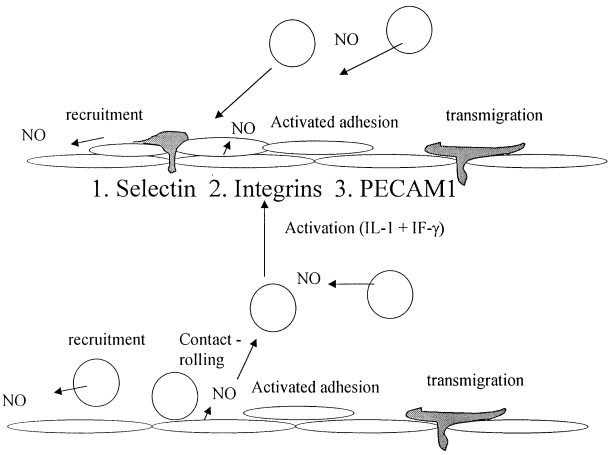

Fig. 1. A representation of basal nitric oxide (NO) actions under 'resting' conditions and in a proinflammatory situation. Bottom: Basal NO is continuously released at low levels. These levels prevent immunocytes from reacting with the vascular endothelium and each other. However, some cells, based on the level of their own activation, may overcome this influence and adhere and possibly undergo transendothelial migration, however the numbers of cells doing this is very small. Top: Once exposed to a proinflammatory stimulus (IL-1 and interferon-gamma), adhesion molecule expression is enhanced in the microenvironment, overcoming basal NO stimulated suppression of an activated state. Thus, we surmise the balance of signaling molecules that can be tipped in either direction regulates the microenvironment, depending on the strength of the stimulus. Additionally, for example, once the balance is tipped toward a proinflammatory situation in time NO levels will again rise, i.e, cNOS in an attempt to reestablish a 'resting' state. In the event cNOS stimulated NO cannot restore this balance, the proinflammatory signals, i.e, cytokines, may then induce iNOS formation as the last attempt. In this last attempt, NO itself by being so reactive may induce tissue damage.

2.1. Cellular mechanisms of NO action

Nitric oxide has been implicated in cellular events in several systems. In this section we critically look at the evidence for a role for this molecule in immune surveillance in two systems: endothelial-leukocyte interaction and in glia; we then focus on NO-mediated events in the nervous system, and finally consider the effects of NO on cell shape.

2.1.1. Immune and vascular systems

One of the key stages in the immune response is the recruitment and activation of leukocytes by the endothelium. Leukocytes, in their interaction with the endothelium, play a role in immunosurveillance; the communication between them constitutes a local regulatory process, which we have called autoimmunovascular regulation, similar in concept to autoimmunoregulation (Fricchione and Stefano, 1994; Scharrer and Stefano, 1994; Stefano, 1989, 1992; Stefano and Scharrer, 1994; Stefano et al., 1992, 1994).

Leukocyte activation by the endothelium occurs in stages (Fig. 2). The initial step is the attraction of the leukocytes to the endothelium. This is followed by increased leukocyte adhesion and change in shape and finally migration across the endothelium (Stefano, 1998). These cellular changes are accompanied by scheduled changes in synthesis of molecules which regulate cell-matrix interactions (Bevilacqua, 1993; Luscinskas and Lawler, 1994; McEver, 1994; Springer, 1994).

Normally, non-activated leukocytes roll along the endothelium. The interaction between the two cell types is loose and reversible and mediated by a family of adhesion molecules known as selectins. Activation of leukocytes occurs in response to the release of several chemoattractants including leukotriene B_4 and interleukin 8 (IL-8). In the presence of these agents immunocytes cease to roll and start to flatten, adhering with greater strength to the endothelial lining. This last aspect of immunocyte behavior is referred to as activation.

Following activation, the leukocytes show increased flattening and adherence to the endothelial matrix (Fig. 2). These changes are mediated by a family of ad-

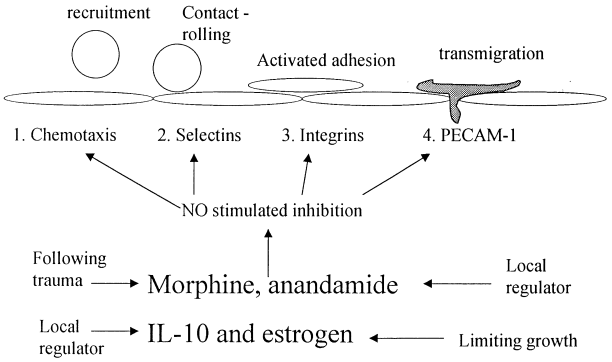

Fig. 2. Representation of morphine, anandamide, interleukin (IL)-10 and estrogen actions on immunocytes, endothelia and their interaction. Top. The interaction of leukocytes with the endothelial lining of blood vessels occurs in stages. Leukocytes can roll across the endothelial surface via weak interactions with selectins following their recruitment. If the proinflammatory signal is strong, i.e, IL-8 presence, the immunocytes cease to roll and start to flatten, adhering with greater strength to the endothelial lining. Once this activation has taken place the next molecules involved in the enhanced adhesion process are the integrins, i.e, ICAM. These surface molecules participate in cell to cell and cell to matrix interactions. Following adhesion, immunocytes may undergo transendothelial migration that may involve PECAM-1, which is present at the junction between endothelial cells as well as on the leukocytes themselves. Bottom: Thus, there is a sequential transition of adherence molecules that come into play. In this regard nitric oxide can inhibit these distinct stages (see text). Agents that induce constitutive nitric oxide synthase, such as the ones shown, have the ability to limit the extent of a proinflammatory event, prevent it in the first place or be involved with its termination. Additionally, they may also keep these cells, depicted in the figure, from becoming too sensitive to stimulation, i.e. basal inhibitory action.

hesion molecules, the integrins, such as ICAM-1 (Bevilacqua, 1993; Luscinskas and Lawler, 1994; McEver, 1994; Springer, 1994). A number of other molecules may also interact with these surface recognition factors; including adhesive glycoproteins, e.g., fibrinogen, and immunoglobulins. Interestingly, immunoglobulins also have the capacity to arrest immunocyte locomotion. There is some evidence that integrin binding may have some degree of cell-type specificity: VCAM-1, which is induced by cytokines, preferentially binds lymphocytes and monocytes (Stefano, 1998). Adherent immunocytes are able to undergo transendothelial migration; this process requires PECAM-1, which is present at the junction between endothelial cells as well as on the leukocytes themselves (Bevilacqua, 1993; Luscinskas and Lawler, 1994; McEver, 1994; Springer, 1994).

In summary, ever increasing concentrations of chemoattractants both guide the leukocytes as well as activate them; the strength of the cell-cell interaction allows the leukocytes to maintain a precise 'footing'. The sequential transition of immunocyte binding from low to high adherence, which allows the immunocytes to pass out of the vessel lumen, is governed by the controlled appearance of a series of molecules also having increased binding capacities: first the selectins, then the integrins, and finally PECAM-1 (Figs. 1 and 2). The process of cell recruitment to a site of antigenic challenge thus appears to be highly regulated: there is evidence for imunocyte selectivity, sequentially timed appearance of molecules and degree of cell activation (Pober and Cotran, 1990; Stefano, 1998; Stefano and Salzet, 1998; Stefano et al., 1998b,c).

2.1.1.1. The role of NO. There is considerable evidence that NO down-regulates the immunocyte-endothelial interaction (Figs. 1 and 2). However, few studies have differentiated between the activities of cNOS and iNOS.

NO has been shown to inhibit platelet and neutrophil aggregation (Kubes et al., 1991) and inhibitors of NOS, e.g., N^G-nitro-L-arginine methyl ester (L-NAME) and L-arginine, increase platelet adhesion (Radomski et al., 1987; Simon et al., 1993) and enhance leukocyte adhesion. This has been demonstrated in venules from a variety of tissues and species, including cat postcapillary venules (Kubes and Granger, 1992; Kubes et al., 1994; Kurose et al., 1993, 1994); rat mesenteric venules (Kubes et al., 1993; Kurose et al., 1993; Suematsu et al., 1994), rat lung venules and cat heart vessels (Bodnar and Pasternak, 1993; Ma et al., 1993). In vitro, NO inhibits monocyte adhesion to porcine aortic endothelial cells (Bath et al., 1991). In human vessels, the adherence of monocytes and granulocytes is reduced following the stimulation of cNOS (Bilfinger et al., 1997; Stefano, 1998; Stefano et al., 1998a,b,c) and, in the presence of NO, monocytes, granulocytes and endothelial cells become round and inactive (Magazine et al., 1996; Stefano et al., 1996a). These findings strongly indicate that NO can diminish the adherence and level of activation of leukocytes and endothelial cells.

2.1.1.2. Glia. NO plays a role in the nervous system's immune response similar to that described in the endothelium-leukocyte interaction. There is considerable evidence that microglia are involved in immune function in the nervous system and that invertebrates have a similar class of cells. Most of the evidence for the role of NO in the invertebrate nervous system is based on work in our laboratory. Molluscs have a distinct subset of glial cells which are closely related to immunoactive hemocytes (immunocytes), and which leave the ganglia following surgical trauma. These cells are morphologically similar to vertebrate microglia (Dobrenis et al., 1995; Sonetti et al., 1994; Stefano et al., 1994).

Both mammals and invertebrates have comparable

mobile immune cells which can enter various tissues, including the nervous system. When monocytes from mammals or invertebrates are restricted to the nervous system, they appear to undergo similar conformational changes and some biochemical dedifferentiation; these changes are somewhat reversible. In *Mytilus*, as the microglia leave the ganglion, their shape progressively changes from stellate to rounded to more or less ameboid; this sequence is similar to that seen in *Mytilus* immunocytes (Sonetti et al., 1994; Stefano, 1989; Stefano et al., 1989a,b).

Microglia from both vertebrates and invertebrates show phagocytic activity, which is characteristic of immunocompetent cells; both mammalian and insect microglia are involved in the removal of degenerating neural tissue (Liu et al., 1996; Sonetti et al., 1994, 1997; Stefano and Scharrer, 1996; Stefano et al., 1994).

Microglia are similar to immune cells in their response to morphine. Morphine, at 10^{-6} M, decreases both the number of cells emerging from excised ganglia and the degree of their transformation to the 'active' ameboid form (Liu et al., 1996; Sonetti et al., 1997; Stefano et al., 1996a). This effect is dose-dependent and naloxone-sensitive. Morphine exerts a similar effect on mobilized microglial cells from the frog *Rana pipiens* (Sonetti et al., 1994).

Finally, microglia and immunocytes have a similar complement of immunoregulatory molecules, which is often species specific. For example, the cytokine IL-1 is found in vertebrate and invertebrate microglia and immunocytes (Paemen et al., 1992; Stefano et al., 1994, 1996a). In *Planorbarius*, a fresh water snail, both immunocytes and microglia have ACTH- and β-endorphin-like substances (Sonetti et al., 1994), whereas the immunoreactivity to these peptides in this cell-type could not be demonstrated in *Mytilus*.

2.1.2. The nervous system

The central nervous system (CNS) is unique in that it uses all three isoforms of NOS to produce NO. The constitutive isoforms, e- and n-NOS, can be demonstrated in the normal CNS; however, iNOS is not expressed in the healthy CNS (Dawson and Dawson, 1995). Pathological states, e.g., trauma, cerebral ischemia, and neuronal diseases increase the levels of e- and n-NOS and, most importantly, induce iNOS (Dawson and Dawson, 1996a,b). The patterns of expression of NOS in the CNS are, therefore, similar to that seen in immune and vascular tissues.

n-NOS is a calcium-calmodulin dependent enzyme (Bredt and Snyder, 1990; Jean et al., 1994; Szabo, 1996) and requires increases in intracellular calcium for activation plus NADPH, tetrahydrobiopterin, O_2, and heme as cofactors. The binding of heme and tetrahydrobiopterin occur following the increase of intracellular calcium; after binding of heme, nNOS monomers loosely dimerize. nNOS is inactivated by phosphorylation by calcium/calmodulin cAMP and cGMP-dependent protein kinases and by protein kinase C (Dawson and Dawson, 1996a,b).

In the CNS, nNOS appears to play a key role in the formation of exhibit markedly cGMP; nNOS-negative transgenic mice have markedly reduced levels of cGMP (Huang et al., 1994). NO-induced changes in cCMP may regulate calcium and sodium channels as well as cGMP-dependent enzymes (Szabo, 1996).

In the CNS, NO may be involved in gene transcription and translation. In neuronal cells, NO amplifies calcium-induced gene transcription and regulates neuronal growth and differentiation (Joseph and Bidlack, 1994; Peunova and Enikolopov, 1993, 1995), NO, derived from nNOS, regulates transcription of several proinflammatory mediators such as IL-6 and TNF-α (Togashi et al., 1997) possible through the inhibition of NF-κB activity in glial cells (see below).

In addition to its role in gene regulation, NO is involved in the modulation of neurotransmitter release. In cultured neurons, exogenous NO evokes the release of [^{3}H]-dopamine (Hanbauer and Grilli, 1992; Hanbauer et al., 1992; Lee et al., 1976). NO is also involved in the release of catecholamines in both the CNS and in peripheral nerves (Deutsch et al., 1997; Rialas et al., 1998) and in invertebrate ganglia (Stefano et al., 1997b); acetylcholine; GABA and neuroactive amino acids (Kuriyama and Ohkuma, 1995; Ohkuma et al., 1995). NO-induced transmitter release has been shown to occur via several mechanisms. Norepinephrine release is dependent on the activation of NMDA receptors by glutamate (Montague et al., 1994); NO-induced GABA release is dependent on Ca^{++} and Na^{+} (Ohkuma et al., 1996b). NO can effect increases in transmitter release both alone and by combining with superoxide to form the compound peroxynitrite (Kieffer et al., 1992; Kimura et al., 1985; Ohkuma et al., 1996a).

2.1.2.1. NO and learning. Of special interest is the finding that NO may play a role in higher-order processes. Two forms of synaptic modulation associated with learning and memory, long-term depression in the cerebellum and long-term potentiation in the hippocampus may be influenced by NO (Dawson and Dawson, 1996a,b; Holscher, 1997). Deletion of the genes for n- and e-NOS in transgenic mice, as well as application of NOS inhibitors such as *N*-nitroso-L-arginine, have been shown to impair spatial learning (Holscher, 1997).

2.1.2.2. Distribution of NO in the nervous system. Depending on the area of the brain, nNOS is co-localized with several neurotransmitters. For example, in

the cerebral cortex, nNOS is co-localized with cells containing somatostatin, neuropeptide Y, and GABA, whereas in the pedunculopontine tegmental nucleus of the brainstem, nNOS is co-localized with choline acetyltransferase, but not with somatostatin or neuropeptide Y (Dawson and Dawson, 1996a,b). N-NOS is found throughout the cerebellum. Cerebellar granule cells release NO after exposure to glutamate agonists (Garthwaite et al., 1988); this activation is linked to increased intracellular calcium due to activation of NMDA receptors. In the cerebellum, nNOS is also found in GABAergic basket cells. In glia, nNOS is the predominant isoform (Togashi et al., 1997). Except for the cerebellum, where all granule cells express nNOS, only 1–2% of neurons in the corpus striatum and cerebral cortex are positive for nNOS (Dawson and Dawson, 1995). In the gastrointestinal and urogenital systems, nNOS is found in selected non-adrenergic, non-cholinergic cells where NO mediates smooth muscle relaxation (Dawson and Dawson, 1995).

2.1.2.3. NO and neurotoxicity. Excessive production of NO and its reaction product peroxynitrite are considered to be responsible for NMDA neurotoxicity (Dawson and Dawson, 1996a,b). Activation of NMDA receptors, a subtype of glutamate receptors, leads to conversion of L-arginine to citrulline and NO. NMDA neurotoxicity can be reduced by NOS inhibitors, removal of L-arginine and binding of extracellular NO by reduced hemoglobin (Dawson and Dawson, 1996a,b; Ho et al., 1992; Kusher et al., 1994). nNOS negative transgenic mice show a marked (50%) reduction of neurotoxicity after intrastriatal injection of NMDA (Ayata et al., 1997); these effects may be due to both lack of NO production by nNOS, and a decreased production of hydroxyl peroxide.

2.1.2.4. i-NOS vs c-NOS and neurotoxicity

The observation that nNOS negative mice have smaller infarct volumes than wild-type mice (Huang et al., 1994) has led to the hypothesis that NO is not neuroprotective. However, it is possible that constitutive NO release may have beneficial effects as it appears to have in vascular tissues (Stefano, 1998, 1999). We should like to consider the hypothesis that effects mediated by NO depend on the amount of NO released into the microenvironment and, that it is the induction of iNOS after ischemia that leads to higher, possibly neurotoxic, NO levels. n-NOS, as a constitutively expressed enzyme, would only release small amounts of NO. In a recent study, exogenous NO was shown to have neuroprotective effects against hydroxyl radical induced neurotoxicity (Mohanakumar et al., 1998). Similarly, in primary cultures of rat mesencephalic dopaminergic cells, NO inhibited peroxide-mediated cytotoxic effects (Wink et al., 1993).

NO neurotoxicity may at least be partially caused by hydroxyl radicals that frequently are produced concomitantly with NO. In systemic inflammatory reactions, as well as in localized inflammation, a proinflammatory phase with release of proinflammatory mediators such as IL-6, IL-8, or TNF-α is followed by an anti-inflammatory reaction dominated by anti-inflammatory substances such as IL-4 or IL-10 (Bone, 1991). We have shown that IL-10 can stimulate cNOS, thereby down-regulating IL-10 activity (Stefano et al., 1997a). Anti-inflammatory mediators are believed to down-regulate an already activated immune system in order to terminate excessive and damaging proinflammatory reactions. IL-10 stimulates endogenous NO release from endothelial cells and inhibits immunocyte activity (Stefano et al., 1997a).

A number of questions about NO in the CNS remain to be answered. The effects of NO released after stimulation of n- or e-NOS in the CNS during inflammatory processes remain to be elucidated. It is also not known if, in the CNS, anti-inflammatory mediators exert their effects via NO release. Furthermore, little is known about the intercellular effects of NO. The current literature on the role of NO in neurodegenerative and inflammatory diseases has focused on its intracellular effects, while its role as an intercellular messenger has been considered mainly in relation to synaptic plasticity. This latter action of NO is in line with its ability to alter the shape of immune and endothelial cells (Magazine et al., 1996; Ottaviani et al., 1993; Stefano et al., 1995, 1996a).

2.1.3. Cell shape

As we have discussed, NO has the ability to alter the shape of various types of cells, i.e., human immunocytes, human monocytes and invertebrate immunocytes, including microglia (Magazine et al., 1996; Ottaviani et al., 1993; Stefano et al., 1995, 1996b). Various endogenous signaling molecules, whose receptors are coupled to cNOS, can bring about a relatively rapid change of shape (Fig. 3). For example, both anandamide, an endogenous endocannabinoid, and morphine exhibit this ability in human and invertebrate immunocytes, microglia and human endothelial cells (Magazine et al., 1996; Ottaviani et al., 1993; Stefano et al., 1995, 1996a). Estrogen can also act via NO to affect cell shape (Prevot et al., 1999; Stefano et al., 1999) (Fig. 3). Acting via a cell surface receptor, estrogen stimulates cNOS to release NO; this initiates cell rounding and inactivity, i.e., down-regulation. Furthermore, once the effect of cNOS derived NO activity (round and inactive) wears off, the cells, for a short period of time, exhibit a simple rebound effect characterized by hyperactivity (Stefano et al., 1998b,c). This suggests, that NO, under some circumstances, can appear to activate cells. This has been recently

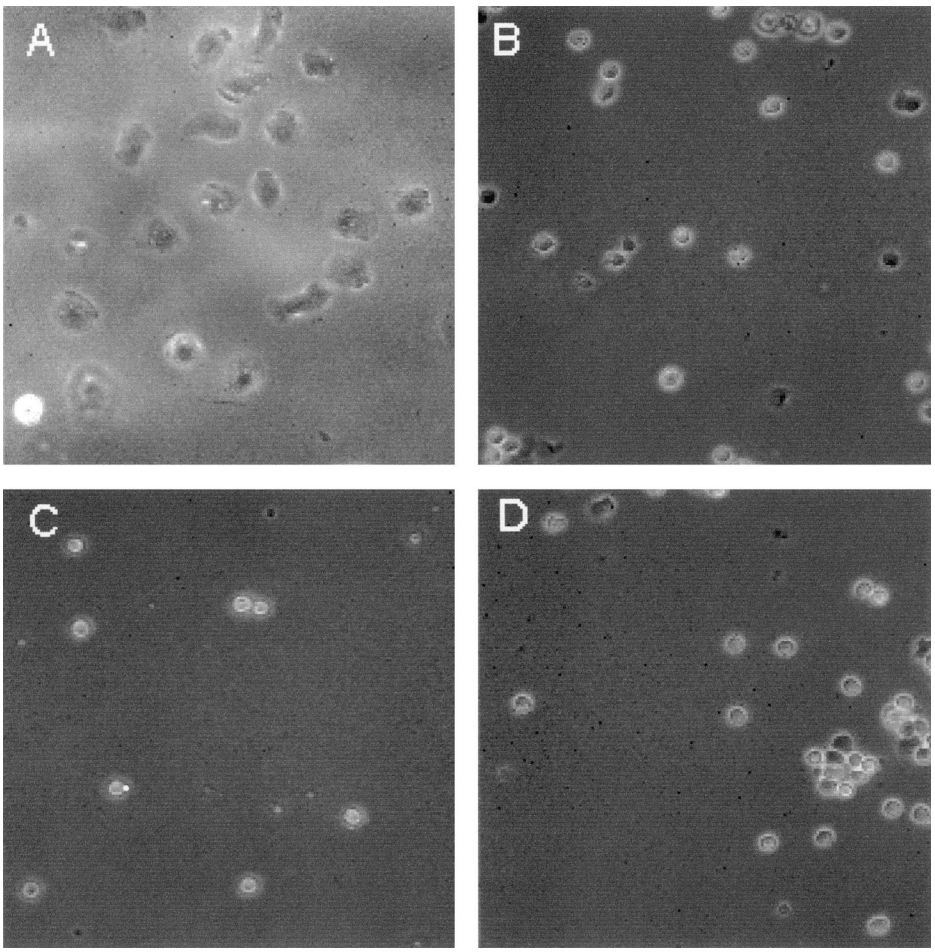

Fig. 3. Exposure of human granulocytes to compounds stimulating cNOS-derived NO initiate cell rounding. (A) Control spontaneously active cells exhibit an ameboid-like shape while moving randomly. Cells from the same source that were exposed to (B) morphine (10^{-6} M), (C) anandamide 10^{-6} M and (D) 10^{-9} M 17-β-estrodiol round up and become inactive within 7 min of this exposure. As noted in the text each of these ligands stimulate NO release from granulocytes that can be antagonized by the respective receptor antagonist and NOS inhibition.

reported by Goligorsky and colleagues for endothelial cells after a prolonged incubation period (Noiri et al., 1997, 1998a,b).

Thus, at different times NO can promote cell shape changes that may represent an activated (mobile) or inactivated (round) cell state. Low, basal levels of NO, tonally expressed, may exert functionally significant constraints on cell shape. The result of this phenomenon is that NO helps to keep microglia stationary in the CNS, thereby potentiating their immunosurveillance activity. This interpretation is consistent with our observation that morphine, via a NO-mediated process, inhibits microglia egress after trauma (Liu et al., 1996). Furthermore, since the NO is derived from cNOS, its action is transient and weak and can be overridden by a stronger activating stimulus (Fig. 1).

NO can also act within the cell to initiate regional shape changes, i.e., cause cells to extend to retract or extend portions of their cytoplasm (Noiri et al., 1997, 1998a,b). This action of NO may be by way of ADP-ribosylation of actin causing actin depolymerization resulting in the inhibition of shape changes by these cells (Clancy et al., 1993; Jun et al., 1996). We can speculate that these intracellular effects on cell shape may be the basis for cellular communication within the median eminence and provide a mechanism for exocytosis into capillary beds (Prevot et al., 1998, 1999a,b).

2.1.4. Molecular mechanisms

2.1.4.1. Adhesion molecules. As we have already discussed, NO inhibits endothelial cell-leukocyte activation. The mechanism of NO action appears to be at the level of various adhesion molecules (Figs. 1 and 2). Sodium nitroprusside, a potent NO donor, inhibits the adhesion of gel-filtrated thrombin stimulated platelets and leukocytes, which is mediated by selectin-P (Dembinska-Kiec et al., 1993). The beta 2-integrin, CD18, and the endothelial cell adhesion molecule ICAM-1 increase the surface adhesion of neutrophils to endothelial cells; this increased adhesion can be blocked by exposure to a NO-donor or to monoclonal antibodies

directed against these molecules (Niu et al., 1995). In a study of postischemic cat mesenteric venules, NO donors decreased polymorphonuclear leukocyte adherence (Kubes et al., 1994) and the authors suggested that NO donors may provide protection from tissue injury by preventing the adhesion of polymorphic nucleocytes. Our previous reports also support this hypothesis (Bilfinger and Stefano, 1996; Bilfinger et al., 1993, 1996, 1997, 1998; Stefano et al., 1994, 1995).

In a recent report, DeCaterina and colleagues (DeCaterina et al., 1995) demonstrated that prior exposure of human saphenous vein endothelial cells to NO decreased the adherence of immunocytes in response to several adhesion factors — IL-1α, VCAM-1, IL-1 β, IL-4, tumor necrosis factor (TNF alpha), or bacterial lipopolysaccharide (LPS). Furthermore, NO also decreased the endothelial expression of E-selectin and secretable IL-6 and IL-8. NO probably acts to repress VCAM-1 gene transcription by inhibiting NF-kappa B (DeCaterina et al., 1995). These studies suggest that the effect of NO on cytokine synthesis may contribute, in part, to its antiatherogenic and anti-inflammatory properties.

2.1.4.2. Morphine and related compounds. We have repeatedly shown that the effects of morphine and anandamide are coupled to NO release in human endothelial cells (Stefano et al., 1995, 1996b, 1998b,c). We have also demonstrated that cNOS derived NO release regulates iNOS activity. Endogenous signaling molecules may use NO to directly inhibit adenylate cyclase activity (Stefano et al., 1998a). In a recent study we demonstrated that activation of human endothelial cells with morphine or anandamide stimulated NO release; NO levels peaked within 5 min and returned to basal levels within 10 min, consistent with cNOS activation. On the other hand, iNOS could be activated only after prolonged (2 h) exposure to LPS and interferon-γ (IFN-γ), but remained significantly elevated over basal levels for 24–48 h. The activation of iNOS could be blocked by preincubation of endothelial cells with morphine, anandamide or the NO donor SNAP prior to, but not after, the addition of LPS + IFN-γ. If the cells were preincubated with NOS inhibitors prior to morphine or anandamide, LPS + IFN-γ were able to induce iNOS. These results suggest that NO itself directly affects iNOS activity. cNOS may exert its effect on iNOS by blocking cAMP. LPS + IFN-γ increases cAMP; pretreatment of cells with morphine, anandamide or SNAP before LPS, blocks this increase. These data suggest that cAMP is required for the induction of iNOS in these cells and that NO may directly impair adenylate cyclase activity, preventing iNOS activation. Our results also demonstrate that cNOS derived NO regulates iNOS expression (Stefano et al., 1998a). This type of feedback inhibition by NO on NOS activity has been reported previously (Assreuy et al., 1993; Griscavage et al., 1993; Ravichandran et al., 1995), suggesting that this phenomenon represents an important control mechanism.

2.1.4.2. NF-κB. NF-κB is a DNA binding factor that is essential for the activation of several inflammatory mediators, e.g., tumor necrosis factor (TNF)-α, IFN-β, IL-8, IL-1β, IL-2, and IL-6 (Blackwell et al., 1996). The NF-κB complex consists of two heterodimers, termed p50 (NF-κB1) and p65 (RelA). Upon its activation, in most types of cells, the NF-κB-inhibitor IκBα is phoshorylated and proteolytically degraded (Blackwell et al., 1996). NF-κB is activated by H_2O_2, oxidative stress produced by TNF-α, IL-1β, and endotoxin (Blackwell et al., 1996). After activation, free NF-κB dimers are directly translocated into the nucleus where they bind to the promoter regions of target genes and induce transcription A ~300-amino acid domain that is required for DNA binding and protein dimerization (Blackwell et al., 1996; Siebenlist et al., 1994) (Fig. 4).

The NF-κB/Rel transcription factor family plays an important role in cytokine-induced gene activation during inflammatory responses or after exposure to

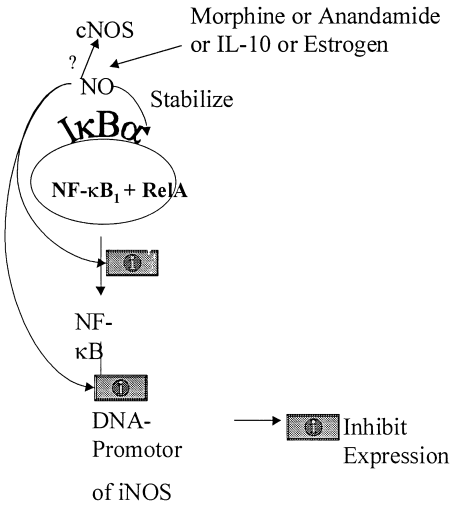

Fig. 4. Representation of endogenous signaling molecules altering NF-κB activation. Briefly, excitatory cytokines, for example, can promote the disassociation of inhibitor IκBα liberating NF-κB which then translocates into the nucleus to bind to specific sites on DNA promotor regions. These DNA promotor regions are coding for proinflammatory cytokines. This binding is then associated with the activation and expression of these proinflammatory associated molecules. Morphine, anandamide, interleukin (IL)-10 and estrogen may alter NF-κB activation. These agents stimulate constitutive nitric oxide synthase (cNOS) liberating nitric oxide (NO). The NO in turn inhibits (i-in box) the disassociation of the IκBα inhibitor complex, NF-κB binding to the respective DNA promotor region and the subsequent expression of the proinflammatory cytokines. This scenario also includes cNOS stimulated NO inhibiting the expression of iNOS as well as cNOS itself (see text and Figure). Thus NO, while exerting global effects, appears itself to be regulated via negative feedback.

bacterial LPS (Siebenlist et al., 1994). The expression of adhesion molecules on the endothelial surface is mediated by TNFα-induced NF-κB activation (Chen et al., 1995; Ledebur and Parks, 1995; Mackay, et al., 1993; Montgomery et al., 1991; Read et al., 1994; Roebuck et al., 1995).

NO inhibits NF-κB transcriptional activation in a variety of cells, including monocytes and endothelial cells (Kupatt et al., 1997; Matthews et al., 1996; Park et al., 1997; Sekkai et al., 1998; Shin et al., 1996; Spiecker et al., 1997) (Fig. 4). In glia, NO derived from nNOS inhibits the transcription factor NF-κB whereas NOS inhibitors increase basal NFκB activation (Togashi et al., 1997). Furthermore, pretreatment of human ramified microglial cells with NO-donors prevents LPS and TNF-α-inducible NO synthesis, probably by inhibiting NF-κB activation which, in turn, inhibits iNOS mRNA expression (Colasanti et al., 1995). In rat peritoneal macrophages, CD23 promotes the nuclear translocation of NF-κB and the transcriptional activation of iNOS (Bayon et al., 1998; Iuvone et al., 1998). NO and various NO-donors induce IκB-α promoter activity and thereby inhibit adhesion molecule expression by an inhibitory effect on NF-κB activation as well (Spiecker et al., 1998).

NO, derived from cNOS, may tonically inhibit NF-κB under non-stimulated conditions. TNF-α, in the presence of two NO donors, was unable to stimulate NFκB from human endothelial cells and NO also stabilized the NFκB inhibitor, IκB-α, by preventing its degradation (Peng et al., 1995). The binding of NF-κB to the NF-κB binding site in the iNOS promoter plays a crucial role in the transcriptional regulation of the iNOS gene (Xie and Nathan, 1994) (Fig. 4). Furthermore, NO donors can directly inhibit the DNA binding activity of NF-κB (Matthews et al., 1996). These findings are supported by a recent study from our laboratory, discussed earlier, in which cNOS-derived NO inhibited iNOS activity induced by LPS and interferon-γ in human endothelial cells (Stefano et al., 1998a).

Inshort, NO derived from cNOS, has the ability to down-regulate a proinflammatory event via the inhibition of NF-κB activation of proinflammatory cytokines. We suggest that the morphine, IL-10, anandamide and estrogen down-regulation of cellular immune function occur by this pathway (Welters et al., 1999). Thus, specific stimuli that initiate cNOS NO release may prevent the appearance of deleterious/protective cytokines. One practical application of this information suggests that by liberating molecules that have the ability to stimulate cNOS and produce a quick burst of NO, the extent and the severity of a pro-inflammatory situation can be limited, for example in sepsis. However, if the proinflammatory event was either initiated earlier or has become extremely strong,

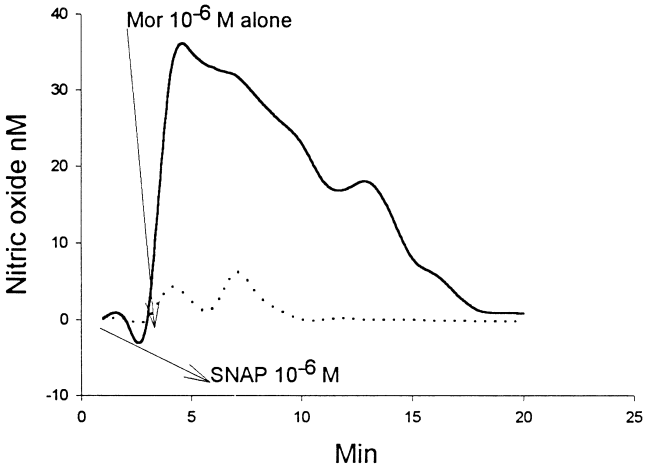

Fig. 5. Real time amperometric nitric oxide (NO) determinations demonstrating morphine (10^{-6} M) stimulated endothelial constitutive NO synthase is inhibited by prior exposure to NO released by the NO donor SNAP (10^{-7} M). SAP, SNAP minus the NO donating ability, does not initiate this inhibition, demonstrating its dependency on NO. The real time determinations are graphed via spline curves. This data indicates that NO exerts an inhibitory negative feedback action on cNOS.

iNOS induction probably cannot be diminished. In this case, iNOS induction may represent a last attempt not only to overcome antigenic challenge but to diminish a strong proinflammatory situation, which could result in biological damage and death.

Interestingly, in past studies we have also demonstrated that the continuous stimulation of cNOS results in diminished NO release as well (Magazine, 1995; Stefano et al., 1998a), suggesting that stimulated cNOS derived NO may also limit its own activity, i.e., autoregulation (Fig. 5). We surmise this from a recent study whereby pressure-stimulated NO release from intact saphenous vein pieces continued to release NO whereas signal molecule stimulated NO release became significantly diminished with time (Bilfinger and Stefano, 1999). Taken together, in experiments where cNOS stimulating agents are continuous or repeatedly exposed to cNOS-receptor coupled cells, in time it would appear that these agents reduce No levels. Clearly, NO signaling is dynamically complex further demonstrating its significance in various biological processes.

2.1.5. Enzymes, hormones and peptides

NO upregulates several enzymes including neutral endopeptidase 24.11, which is also known as CALLA (acute lymphoblastic leukemic antigen) or CD10 (Salzet et al., 1997). Interestingly, neuroscientists refer to the same enzyme as enkephalinase (see Turner et al., 1994). As the name implies, enkephalinase processes proenkephalin and proopiomelanocortin to release enkelytin, an antibacterial peptide, and enkeph-

alin, an endogenous opiate (Salzet et al., 1997; Salzet and Stefano, 1997; Stefano et al., 1998e). Thus, cNOS derived NO stimulates an enzyme that can process protein gene products. These results imply a profound link between the signaling processes involving NO and naturally occurring antibacterial peptides. NO controls and regulates enzymes that are responsible for liberating these crucial molecules that have a proactive protective function (Stefano et al., 1998e).

We have demonstrated that morphine, anandamide and estrogen can stimulate NO release in the vasculature of rat median eminence (Prevot et al., 1998, 1999), providing a role for NO in neurotransmitter release (Stefano et al., 1997b), Morphine and NO, derived from cNOS, release growth hormone and ACTH from these brain fragments; these neuropeptides are involved in the response to stress. Thus, NO is involved in vasodilation; antibacterial and antiviral responses of the host, signal molecule release and the inhibition of immunocyte adherence to the endothelium. NO even down regulates a potential pro-inflammatory response that requires communication in the endothelial-immunocyte microenvironment (Figs. 1 and 2).

3. Tonal nitric oxide

What is the evidence for a basal or tonal level of NO? As noted in several of the above sections of this review, although there appears to be a tonal, or basal, level of NO, much of the evidence is circumstantial. In a series of studies, we measured what we believe is basal NO. In these studies we used an amperometric probe to measure NO in endothelial cells, monocytes, granulocytes and invertebrate ganglia and immunocytes (Magazine et al., 1996) (Fig. 6). Under non-stimulated conditions, the level of NO appeared as a straight line that we 'zeroed' our probe system to; addition of L-NAME (100 mM) resulted in a small drop in the straight line, indicating that a low level of NO was being released in these preparations. No shift was seen in incubation medium without tissue but with L-NAME. Therefore, based on this observation, it is reasonable to conclude that NO was produced in these tissues at very low levels. Based on the information presented in the earlier sections of this review, we believe that this tonal NO is physiologically significant. We speculate that tonal NO tends to diminish the natural excitatory nature of these cells and in so doing provides for a threshold level of stimulation that activating signals need to overcome. Once a threshold level of stimulation occurs, it simply 'tips' the balance of signal molecule strength in favour of excitation. In other words, excitation occurs by disinhibition (Fig. 2).

Current work in our laboratory supports this view. Endothelia, from non-insulin dependent diabetics do not exhibit a tonal level of NO (unpublished; Fig. 6) and in these individuals vascular disease causes disability and death (Catalano et al., 1997). A number of authors, including us, have attributed vascular disease, in part, to alterations associated with eNOS-derived NO. Some have speculated this also may be due to enhanced free radical generation (Nitenberg et al., 1997). Indeed, decreases in the basal level of NO may also contribute to enhanced platelet function as well as neuropathies (Huszka et al., 1997; Pitei et al., 1997).

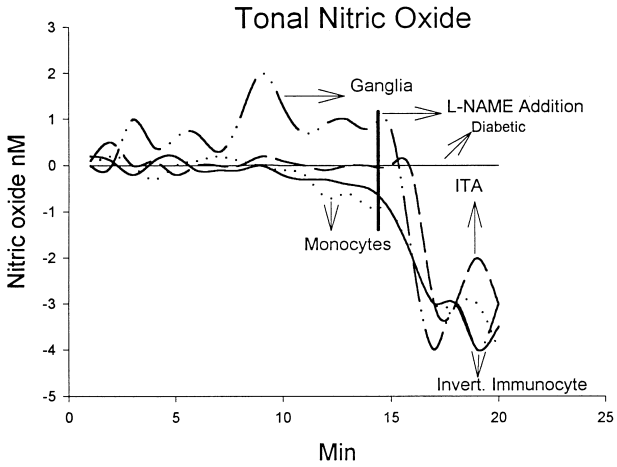

Fig. 6. Real time amperometric nitric oxide (NO) determinations demonstrating basal NO release. The amperometric NO probe is placed in the respective medium containing the following: 1. invertebrate ganglia (n = 10, ganglia); 2. internal thoracic artery segments (ITA, 2, 3 mm sections); 3. human monocytes 10^7/ml; 4. invertebrate immunocytes (10^7/ml) and 5. ITA from a chronic non-insulin dependent diabetic. It can be observed that these living cells produce a base line level of NO, as noted by the line (all plotted as spline curves for better visualization) wavering. In this regard, in these experiments, this line has been set to zero since it is quite stable under resting conditions. However, it can clearly be observed that the addition of L-NAME 100 mM (BAR), a potent nitric oxide synthase inhibitor, lowers basal release even more, thus graphically demonstrating its presence. In the diabetic tissue no drop occurs, indicating functional impairment of the endothelia to generate basal NO.

Taken together, it would appear that tonal NO is quite important in limiting the degree of excitation of nervous, immune and vascular tissues. Furthermore, this tonal NO may manifest itself via effects on adhesion-mediated processes via NF-κB. Other signal cascade mechanisms may also be involved (Fimiani et al., 1999b). Estrogen may exert its beneficial vascular protective actions via these processes as well since it also releases NO from cNOS (Prevot et al., 1999; Stefano et al., 1999). Strengthening this hypothesis is the finding of the cannabinoid CB1 receptor type on mammalian endothelial cells (Deutsch et al., 1997; Bilfinger et al., 1998) and the finding of a partially sequenced mu opiate receptor on human vascular endothelial cells (Stefano et al., 1998d; Fig. 7). In this regard, because this represents such a low level of NO release, it may take years to manifest itself into a

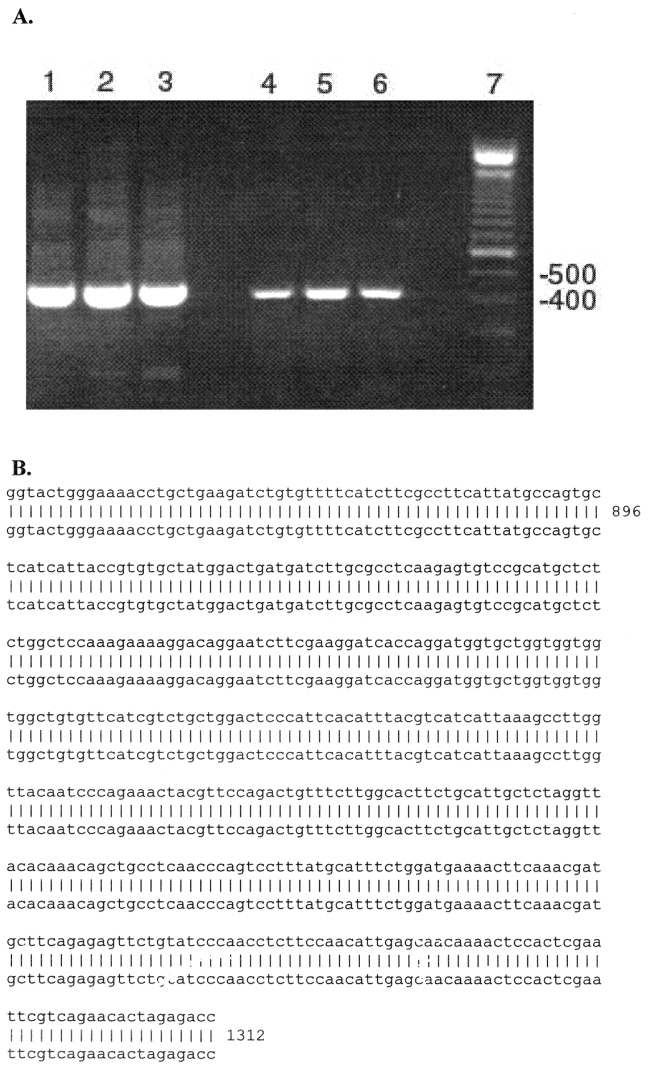

Fig. 7. Sequencing of mu opioid receptor cDNA obtained from human saphenous vein and atrial endothelial cells. Total RNA isolated from these tissues was reverse transcribed and PCR amplified with G3PDH control or with mu specific amplimers. (A) Ethidium bromide agarose gel of reverse transcribed and PCR–amplified fragments. Lanes 1–3, G3PDH amplified fragments from vein, vein treated with 30 ng/ml of IL-1 α/β, and atria, respectively. There appears to be a 40% increase (Gel-Pro Software; Media Cybemetrics, Inc., MD) in expression of the mu transcript following cytokine stimulation, indicating a regulatory role for these signals. Expected product size from the G3PDH amplification is 451 bp. Lanes 4, 5, fragments amplified from vein, and vein treated with 30 ng/ml of IL-1 α/β. Lane 6, fragment amplified from atrial endothelial cells. Expected product size from the mu amplification is 441 bp. (B) Comparison of the putative mu conserved sequence from vein and heart tissues (top) with the known human brain mu opioid receptor sequence (bottom). Both negative and positive strands were sequenced, however, only the sequence from the negative strand is shown. The sequences obtained from the two tissues were identical. Both tissues were washed extensively with PBS to limit the amount of leukocyte contamination as also determined by microscopic examination.

recognizable disorder. In part, this may also be due to the fact that during these years cNOS can be stimulated by other signaling molecules, providing for a limited short-term presence of NO.

4. Clinical and functional implications of NO activity

4.1. Morphine

Morphine and NO seem to be coupled in several processes other than those noted above. Peripheral morphine analgesia involves NO-stimulated increases in intracellular cGMP (Ferreira et al., 1991) and NO has been associated with antinociception (Duarte and Ferreira, 1992) as well as tolerance and dependence (Bhargava, 1995; Dambisya and Lee, 1996; Kolesnikov et al., 1993; Majeed et al., 1994; Vaupel et al., 1995a,b; Xu and Tseng, 1995). NO is involved in the morphine-induced suppression of splenic lymphocyte proliferation (Fecho et al., 1994) and morphine and NO have been linked in the regulation of gastrointestinal function (Gyires, 1994) and food intake (Calignano et al., 1993). Coincubation of invertebrate microglia with L-arginine or the superoxide scavenger, superoxide dismutase, result in significantly higher NO levels following morphine stimulation (Liu et al., 1996). The data suggest that morphine induced-NO release has been conserved during evolution and that it participates in the regulation of cellular conformation and degree of activation.

We have recently shown that endothelia from human blood vessels express μ opiate receptors, indicating that signaling in these cells can be regulated by NO (Stefano et al., 1998d; Fig. 6). Opioid peptides antagonize the morphine-stimulated release of NO derived from cNOS (Stefano et al., 1998d). Thus, at the level of the endothelial cell, opiate alkaloids and opioid peptides have antagonistic actions affecting NO release.

4.2. Cannabinoids

Invertebrate immunocytes and microglia have stereoselective binding sites for anandamide, a naturally occurring cannabinoid (Stefano and Salzet, 1996; Stefano et al., 1996b). This binding site is coupled to NO release, both in invertebrate tissues and in human monocytes. The cannabinoid stimulated release of NO initiates cell rounding; this is similar to the effect of opiate alkaloids. These signaling systems utilize the same effector system, i.e., NO release, but by separate receptors (Stefano and Salzet, 1996; Stefano et al., 1996b). Anandamide signaling has been demonstrated in rat blood vessels where it can also cause vasodilation (Deutsch et al., 1997). In this report, CB1, an

endocannabinoid receptor, and anandamide were also found, indicating that this signaling may involve autovascular regulation, since the entire cannabinoid system is found in this one location.

Tetrahydrocannabinol (THC) inhibits macrophage cell contact-dependent cytolysis of tumor cells (Burnette-Curley and Cabral, 1995). THC also appears to alter antigen processing (McCoy et al., 1995) and the expression of select proteins whose induction is associated with macrophage activation as well as the expression of TNF (Cabral and Fischer-Stenger, 1994; Zheng et al., 1992). THC increases the bioactivity of IL-1 in cultures of mouse resident LPS-stimulated peritoneal macrophages (Shivers et al., 1994). Incubating P388D1 macrophage cells with THC results in a dose-dependent inhibition of cell proliferation, DNA synthesis and phagocytosis (Tang et al., 1992). In earlier reports, THC was shown to inhibit human peripheral blood macrophage spreading and phagocytosis of yeast (Friedman et al., 1986; Specter et al., 1991a,b,c). The inhibition of cell spreading is in agreement with observations made by others and our group (Clancy et al., 1993; Friedman et al., 1986; Jun et al., 1996; Specter et al., 1991a; Stefano et al., 1996a,b) suggesting that, as with morphine, anandamide receptor coupling to NO may be the mechanism initiating cell rounding (Magazine et al., 1996; Ottaviani et al., 1993). Thus, naturally occurring cannabinoids and opiate alkaloids may utilize the same NO-producing effector system. Additionally, anandamide-coupled NO release, on a dose for dose basis, releases less NO in human monocytes, endothelia and invertebrate immunocytes, than morphine (Stefano et al., 1996b). In line with the significance of a low level NO presence, Stefano and colleagues suggested this may be the reason why it is harder to document immune suppressive THC actions as well as addiction compared to these actions with respect to morphine (Stefano et al., 1996b).

4.3. Estrogen

NO is involved in regulating neuroendocrine secretion from the median eminence (ME), and, therefore, plays a role in controlling several adenohypophysiotropic systems. ME capillaries release NO in response to 17β-estradiol; the response to estrogen is immediate, specific to 17β-estradiol and inhibited by the antiestrogen tamoxifen (Prevot et al., 1999a). 17β-estradiol conjugated to bovine serum albumin (E_2-BSA) also stimulated NO release, suggesting mediation by a membrane surface receptor.

Estradiol-induced NO can stimulate GnRH release; this effect is inhibited by inhibitors of NO activity such as hemoglobin (a NO scavenger), L-NAME and L-NIO (nitric oxide synthase (NOS) inhibitors). L-NIO, specific for eNOS, was significantly more potent, suggesting that the estradiol-stimulated NO release arose from vascular endothelial cells. The NO-stimulated GnRH release occurred via guanylyl cyclase activation in GnRH nerve terminals and was abolished by ODQ, a potent and selective inhibitor of NO sensitive guanylyl cyclase. Thus, at physiological concentrations, 17β-estradiol may have immediate actions on ME endothelial cells via non-genomic signaling pathways (Prevot et al., 1999a). These results only further demonstrate the physiological significance of NO derived from cNOS, as well as neurovascular signaling.

Estrogen replacement therapy has been associated with a reduction of cardiovascular events in postmenopausal women. The mechanism that mediates this apparent benefit remains unclear. However, in association with the arguments advanced in this review, it may be related to diminished NO capacity due to estrogen levels dropping in these women. We investigated the acute effects of physiological levels of estrogen on NO release from human internal thoracic artery (ITA) endothelia and arterial endothelia in culture (Stefano et al., 1999). We tested the hypothesis that estrogen acutely stimulates cNOS activity in human endothelial cells by acting on a membrane receptor. NO release was measured in real time with an amperometric probe. Exposure of ITA endothelia and arterial endothelia in culture to 17β-estradiol stimulated NO release within seconds; the release was concentration-dependent, specific for 17β-estradiol and 17β-estradiol conjugated to bovine serum albumin (E_2-BSA), inhibited by tamoxifen, and dependent on the level of intracellular calcium (Stefano et al., 1999). The effect of 17β-estradiol on endothelial NO release was abolished by leeching the intracellular calcium stores. We concluded that estrogen, in physiological doses, acutely stimulates NO release from human endothelial cells via the activation of an estrogen surface receptor that is coupled to increases in intracellular calcium.

As with morphine and endocannabinoids, estrogen down-regulates immunocyte functions, i.e.. chemotaxis and phagocytosis (Al-Afaleq and Homeida, 1998; Boulanger and Luscher, 1990; Garzetti et al., 1998; Ito et al., 1995; Magnusson, 1991; Masana et al., 1990; Mefford et al., 1991; Merrill et al., 1989; Miyagi et al., 1992; Nathan and Chaudhuri, 1997; Okada et al., 1997; Wessendorf et al., 1998). Monocytes express estrogen receptor mRNA as well as an estrogen receptor binding site (Ben-Hur et al., 1995; Suenaga et al., 1996, 1998; Wada et al., 1992) The actions of estrogen can also can be initiated by inhibiting the adhesion potential of the immunocytes and endothelial lining of the vasculature (Mohankumar et al., 1991; Nathan and Chaudhuri, 1997; Saville et al., 1994; Squadrito et al., 1997; Suzuki et al., 1997; Weissman et al., 1995; Yamada et al., 1996).

The coupling of estrogen to cNOS-derived NO is clearly significant. Both estrogen and NO decrease immunocyte adhesion, decrease the vascular endothelium's capability to cause the adherence of immunocytes, and down-regulates immunocytes both before and after proinflammatory events (Stefano, 1998; Stefano et al., 1996a). Estrogen can be thought of then, as acting in parallel with endogenous morphine and the endocannabinoid anandamide (for review, see Stefano, 1998; Stefano et al., 1996a).

4.4. AIDS

Morphine and the human immunodeficiency virus envelope protein gp120 have similar effects on the vascular endothelium. Chronic exposure to morphine enhances the endothelium's ability to hold and activate immunocytes. Because of this, individuals with HIV should have a greater viral load in tissues, e.g, neural tissue (Stefano et al., 1998b,c). Laboratories, including our own, examining a link between drug abuse, opiate exposure, and HIV have long assumed that there is a detrimental link in regard to tissue damage and viral load of the two; however, epidemiological studies do not bear this out (Donahoe and Vlahov, 1998; Vlahov et al., 1991, 1998). In a recent review, speculated that acute exposure of granulocytes, macrophages and vascular endothelial cells to morphine may be beneficial since it results in cNOS NO release and NO is a potent antiviral agent (see Stefano, 1999).

5. Conclusion

It is safe to conclude that in many tissues there is a basal level of NO and that the actions of several signaling molecules require increases in cNOS-derived NO. The large literature on NO and the immune axis may give the impression that these systems are redundant. However, we believe that each signaling system performs this common function, i.e., cNOS derived NO release, under different circumstances. Morphine-coupled NO release, seen after trauma/inflammation, dampens these processes in neural and immune tissues (Stefano, 1998; Stefano and Scharrer, 1994; Tonnesen et al., 1998). Anandamide, by being part of the always present arachidonate and eicosanoid signaling processes, serves to maintain tonal NO in vascular tissues (Fimiani et al., 1999b). We surmise that estrogen provides an extra degree of immunocyte and vascular down regulation in females (Stefano et al., 1999) which may be important in modulation of the immune and vascular trauma associated with cyclic reproductive activities, i.e., endometrial buildup. Given the high degree of proliferative growth capacity during estrogen peak levels in this cycle, NO may function to enhance down regulation of the immune system to allow for these changes. In this regard, it is not difficult to understand the reports documenting various cancers with blocking estrogen actions and conversely reports documenting its anti-cancer protective actions (see Service, 1998).

Given the subtle nature of basal/tonal NO's actions, it will be hard to document its significance over the short term. However, over the long term we already may be observing its effects in progressively debilitating conditions such as diabetes. In other chronic conditions involving inflammatory events such as AIDS-associated neuropathies we may also be observing conditions when tonal NO is absent, leading to enhanced tissue damage. It is hoped this review will provide an impetus for studying the effects of long-term tonal NO release and its biomedical significance.

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