Review

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Supraspinal Circuitry Mediating Opioid Antinociception: Antagonist and Synergy Studies in Multiple Sites

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Key Words

Opioid antinociception · Periaqueductal gray, ventrolateral · Medulla, rostral ventromedial · Locus coeruleus · Amygdala

Abstract

Supraspinal opioid antinociception is mediated by sensitive brain sites capable of supporting this response following microinjection of opioid agonists. These sites include the ventrolateral periaqueductal gray (vlPAG), the rostral ventromedial medulla (RVM), the locus coeruleus and the amygdala. Each of these sites comprise an interconnected anatomical and physiologically relevant system mediating antinociceptive responses through regional interactions. Such interactions have been identified using two pharmacological approaches: (1) the ability of selective antagonists delivered to one site to block antinociception elicited by opioid agonists in a second site, and (2) the presence of synergistic antinociceptive interactions following simultaneous administration of subthreshold doses of opioid agonists into pairs of sites. Thus, the RVM has essential serotonergic, opioid, cholinergic and NMDA synapses that are necessary for the full expression of morphine antinociception elicited from the vlPAG, and the vlPAG has essential opioid synapses that are necessary for the full expression of opioid antinociception elicited from the amygdala. Further, the vlPAG, RVM, locus coeruleus and amygdala interact with each other in synergistically supporting opioid antinociception.

Research over the past 20 years has indicated that supraspinal opioid antinociception is mediated in part by neurons originating in the midbrain ventrolateral periaqueductal gray (vlPAG), which synapse in the rostral ventromedial medulla (RVM) which includes the nucleus raphe magnus (NRM), nucleus reticularis gigantocellularis (NRGC), and NRGC pars α, and then which project to the substantia gelatinosa of the spinal cord [see reviews 7, 25]. The vlPAG appears to support μ-receptor-mediated opioid antinociception [(e.g.) 14, 24, 78]. The RVM supports antinociceptive responses after intracerebral administration of opiate agonists [(e.g.) 14, 43, 74], and their physiological firing characteristics appear to predict the occurrence of antinociception after opiate administration [see review 26]. Direct projections between the vlPAG and NRGC and between the vlPAG and NRM have been described [1, 12, 89, 90], with the latter pathway containing serotonin, enkephalins, neurotensin and substance P [8, 10]. These projections from the vlPAG are quite selective in terms of anatomy and function, and are differentiated from those emanating from other lateral, dorsal and dorsolateral PAG regions [5, 11, 16]. The vlPAG also

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projects to pontine and medullary cell groups (A5, A6, A7) that possess spinally projecting noradrenergic neurons [17, 18, 20, 65] with the densest vlPAG projections [4, 22] found in A6 [locus coeruleus, 13, 14, 76] and A7 [93, 94] neurons capable of supporting antinociceptive responses. The RVM in turn sends highly specific and physiologically relevant projections to the locus coeruleus and immediately surrounding regions [3, 19]. In addition to these brainstem structures involved in opioid antinociception, the amygdala also supports opioid antinociceptive responses based upon intracerebral microinjection [39, 67] and lesion [50, 51] studies presumably through connections between the amygdala and the vlPAG [9, 48]. Thus, a series of supraspinal structures that support opioid antinociception are linked anatomically and physiologically into a potential functional system that mediates the control of responses to nociceptive input.

The purpose of this review is to provide evidence for functional interactions between pairs of these sites, and identify the neurochemical substrates of such mediation. Two approaches have been employed by our and other laboratories to establish whether opioid antinociceptive responses elicited from one site are either mediated by and/or interacted with opioid antinociception elicited from a second site. The first approach described in this review is to determine whether opioid antinociception elicited from one site is blocked by the prior administration of general or selective antagonists into a second site. This antagonist approach employed by our laboratory addresses several important issues. First, the studies are performed using full dose-response curves of the antagonists to evaluate changes in the full time-response curves of the agonists. Second, two nociceptive tests are employed to study the generalizability of the results. The tail-flick test [21] is a measure of reactivity to noxious heat, and is mediated at the level of the spinal cord based upon anatomical evidence [31]. The jump test [23] measures reactivity to noxious shock, and is mediated by supraspinal and suprasegmental mechanisms. Third, the site specificity of the antagonist effects is routinely assessed by assessing the capability of the antagonists administered into control placements dorsal or lateral to the intended sites to alter opioid agonist-induced antinociception from the first site. Fourth, a given antagonist administered into one site may vitiate the antinociceptive response of opioids administered into a second site by merely producing a corresponding hyperalgesic response. Therefore, the effects of these antagonists upon basal nociceptive thresholds are routinely examined. Finally, in some studies, agonist-induced specificity was assessed by testing more than one opioid agonist in a given site. A second approach to assess functional relationships between sites mediating opioid antinociception is synergy which was elegantly established between spinal and supraspinal opioid systems by Yeung and Rudy [95], and subsequently characterized by others [68, 69]. The present review will examine the nature of synergistic antinociceptive interactions between sites using multiple opioid agonists.

Opioid Agonists in the vlPAG and Serotonergic Antagonists in the RVM

The direct projections between the vlPAG and the NRM [1, 12] appear to contain serotonin in 55–63% of the fibers [10]. Further, autoradiography confirmed serotonin receptors on RVM neurons, including those of the $5HT_2$ and $5HT_3$ subtypes [62, 92] which have been implicated in antinociceptive processes per se [32, 58]. Moreover, inactivation of the RVM by localized microinjections of lidocaine blocks morphine antinociception elicited from the vlPAG [30, 65, 88]. Therefore, our first series of studies [44, 45] examined the neurochemical substrates of this relationship between the RVM and morphine antinociception elicited from the vlPAG by determining whether pretreatment of either general (methysergide), $5HT_2$ (ritanserin) or $5HT_3$ (ICS205930) serotonin receptor antagonists into the RVM would alter morphine antinociception elicited from the vlPAG. Morphine at a dose of 2.5 μg in the vlPAG elicited a potent antinociceptive response on the tail-flick and jump tests (fig. 1). Pretreatment with the general 5HT receptor antagonist, methysergide, at doses between 0.5 and 5 μg in the RVM significantly reduced morphine antinociception elicited from the vlPAG on the tail-flick (61%) and jump (58%) tests (fig. 1, top panels). Pretreatment with the selective $5HT_2$ antagonist, ritanserin, in the RVM produced significant dose-dependent inhibition of morphine antinociception elicited from the vlPAG on the tail-flick (80%) and jump (61%) tests (fig. 1, middle panels). Finally, pretreatment with the selective $5HT_3$ antagonist, ICS205930, in the RVM produced significant dose-dependent inhibition of morphine antinociception elicited from the vlPAG on the tail-flick (88%) and jump (61%) tests (fig. 1, lower panels). These antagonist effects appeared to be selective to opioid antinociception since basal nociceptive thresholds failed to be altered following RVM microinjections of either methysergide, ritanserin or ICS205930. These antagonist effects also appeared to be site-specific since administration of these antagonists into misplaced medullary sites

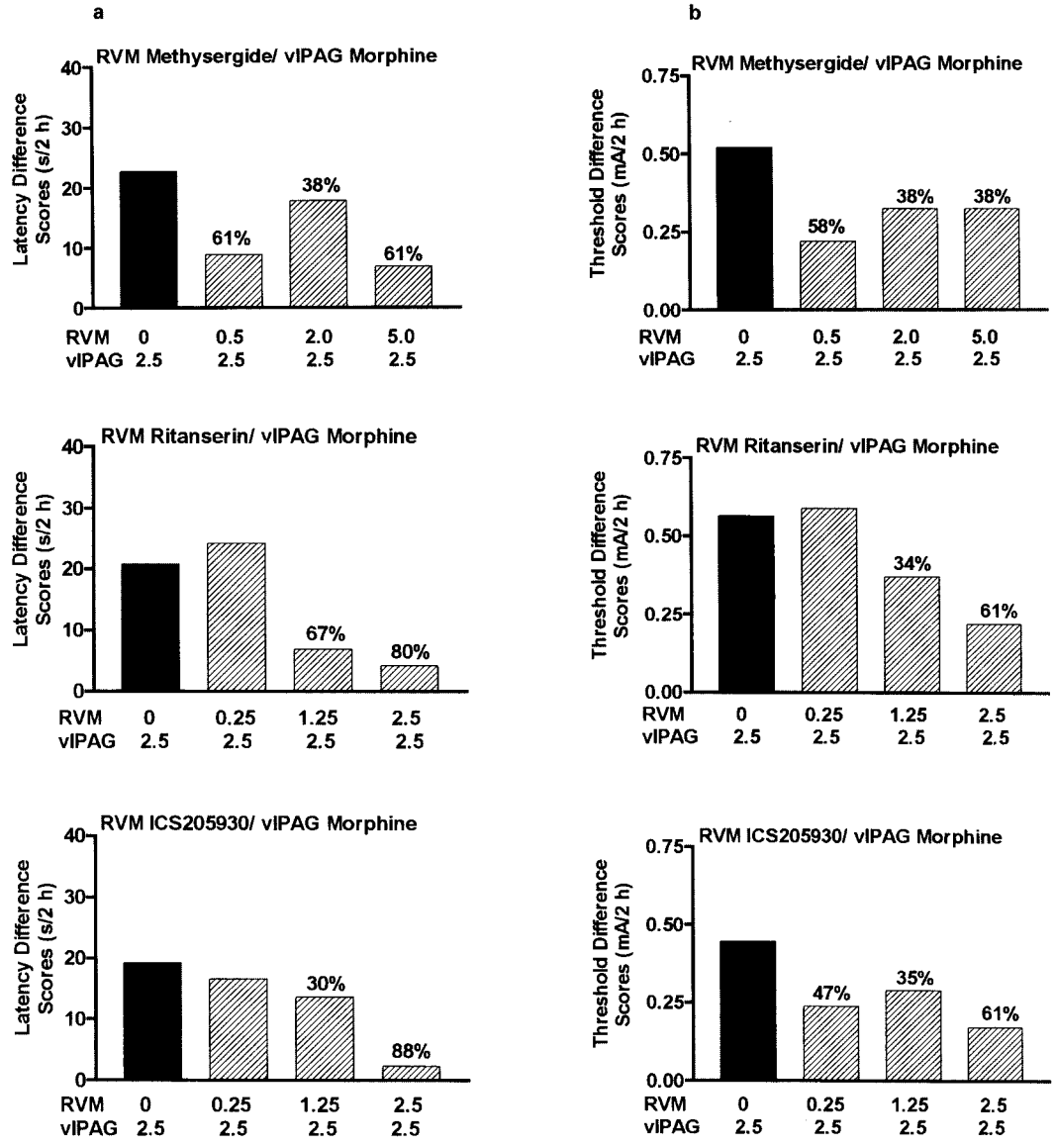

Fig. 1. Alterations in morphine antinociception on the tail-flick (**a**) and jump (**b**) tests elicited from the vlPAG following pretreatment with general (methysergide), $5HT_2$ (ritanserin) or $5HT_3$ (ICS205930) sertonergic antagonists in the RVM. All dose values in this and subsequent figures are in micrograms.

lateral and/or dorsal to the RVM failed to alter morphine antinociception elicited from the vlPAG.

The RVM appears to contain two types of cells involved in nociceptive processing: ON cells increase firing just prior to the occurrence of a tail-flick response, while OFF cells pause in their firing prior to a tail-flick response [26]. Whereas morphine administered into the vlPAG inhibits medullary OFF-cell firing [6, 56], opioid activation of medullary OFF cells is thought to occur through disinhibition of GABA-containing interneurons [37, 53, 77]. In analyzing the relationship, if any, between serotonin immunoreactivity and RVM physiological cell types, it was found that most serotonin immunoreactivity in the RVM was found in NEUTRAL cells [63, 64], the firing rates of which fail to be affected by opioid administration [26]. Such cells are found in the NRM and ventral NRGC

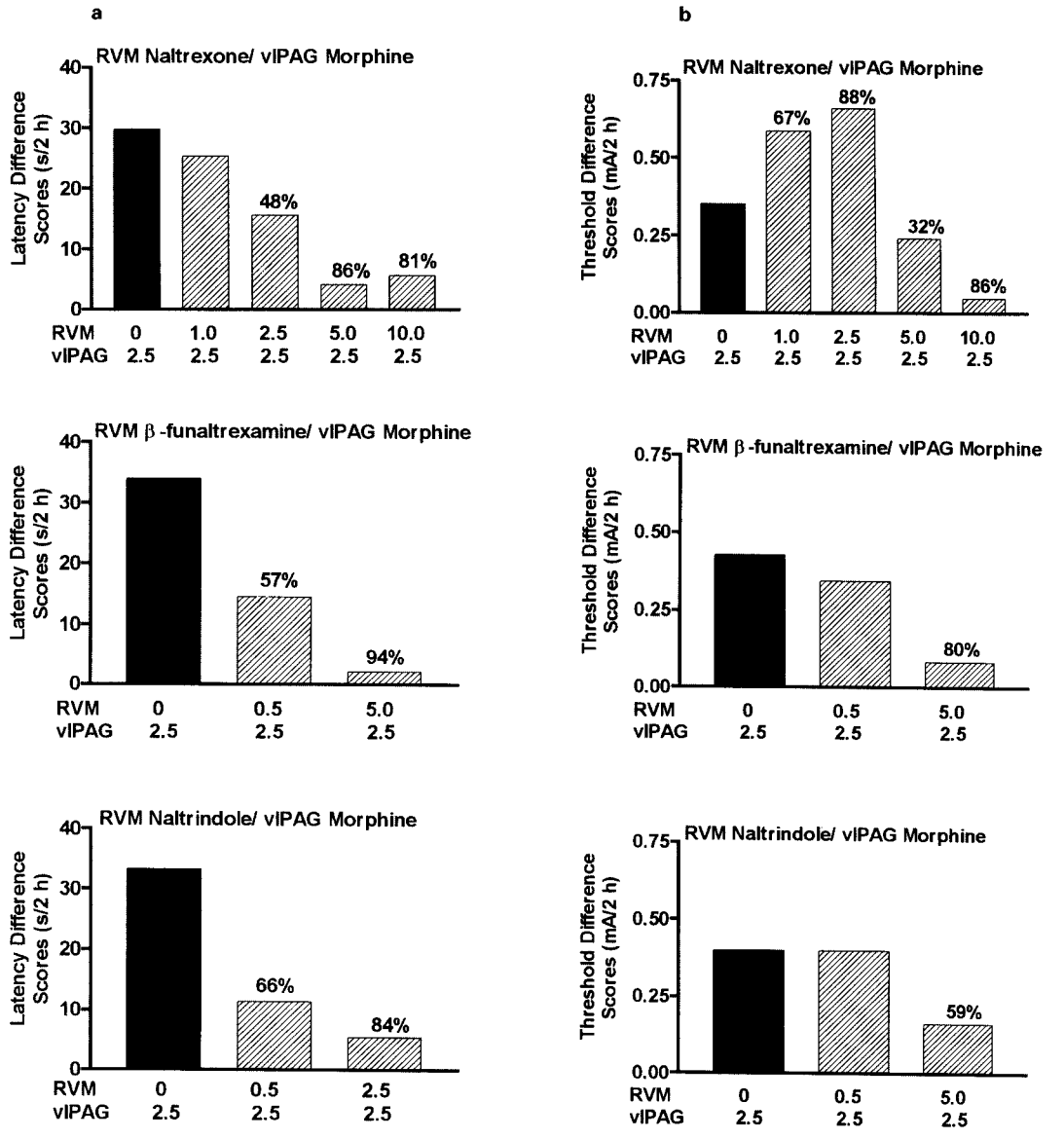

Fig. 2. Alterations in morphine antinociception on the tail-flick (**a**) and jump (**b**) tests elicited from the vlPAG following pretreatment with general (naltrexone), μ (β-funaltrexamine)- or δ (naltrindole)-opioid antagonists in the RVM.

[29], and appear to have a slow, steady discharge, suggesting tonic rather than phasic modulation of spinal processes [52]. These physiologically identified serotonergic cells in the RVM failed to respond during antinociception elicited by either electrical stimulation of the vlPAG [28] or systemic morphine [27]. These data argue that cells containing serotonin as a neurotransmitter in the RVM do not appear to be integral in the mediation of supraspinal opioid antinociception. The antagonist data described above indicate that blockade of serotonergic receptors in the RVM prevent the full expression of morphine antinociception elicited from the vlPAG. It is important to note that those cells that have serotonin receptors can be distinct from those that display serotonin immunoreactivity, and this factor can explain the potential discrepancy between the functional and physiological data. Indeed,

iontophoretic application of serotonin in the RVM appeared to facilitate activity of all three physiologically described classes of neurons [73], and it would be important to determine if selective serotonin subtype agonists would produce more selective physiological actions.

Opioid Agonists in the vlPAG and Opioid Antagonists in the RVM

The direct projections between the vlPAG and the NRM [1, 12] also appear to contain enkephalin [8]. Therefore, our second series of studies [46] examined whether pretreatment of either general (naltrexone), μ (β-funaltrexamine: β-FNA)- or δ (naltrindole)-opioid receptor antagonists into the RVM would alter morphine antinociception elicited from the vlPAG. The potent antinociception elicited by morphine in the vlPAG was significantly and dose-dependently reduced by RVM pretreatment with naltrexone on the tail-flick (81%) and jump (86%) tests (fig. 2, top panels). RVM pretreatment with the selective μ-opioid antagonist, β-FNA, produced significant dose-dependent inhibition of morphine antinociception elicited from the vlPAG on the tail-flick (94%) and jump (80%) tests (fig. 2. middle panels). Finally, RVM pretreatment with the selective δ opioid antagonist, naltrindole, produced significant dose-dependent inhibition of morphine antinociception elicited from the vlPAG on the tail-flick (84%) and jump (59%) tests (fig. 2, lower panels). These antagonist effects appeared to be selective to opioid antinociception since basal nociceptive thresholds failed to be altered following RVM microinjections of either naltrexone, β-FNA or naltrindole. These antagonist effects also appeared to be site-specific since administration of these antagonists into misplaced medullary sites lateral and/or dorsal to the RVM failed to alter morphine antinociception elicited from the vlPAG. Other neuroanatomical loops have been identified indicating potential alternative opioid-dependent pathways mediating morphine antinociception elicited from the vlPAG. Naloxone administration into the habenula significantly reduced morphine antinociception elicited from the vlPAG, and naloxone administration into the nucleus accumbens significantly reduced morphine antinociception elicited from the habenula [49].

Opioid antagonists in the RVM also appear to be responsible for other antinociceptive actions in the vlPAG since antinociception elicited by the $GABA_A$ receptor antagonist, bicuculline, in the vlPAG is significantly reduced by RVM pretreatment with either general (naltrexone) or μ-selective (CTOP) opioid antagonists [72]. Importantly, these antagonists were ineffective in reducing bicuculline antinociception when they were injected into misplaced medullary placements. A parallel study showed that administration of either morphine or bicuculline into the vlPAG significantly reduced ON-cell firing in the RVM, which was reversed by iontophoretic application of naloxone in the RVM [57]. Whereas bicuculline in the vlPAG increased OFF-cell firing in the RVM, this effect was unaffected by naloxone. Further, the μ-selective opioid agonist, DAMGO, in the RVM depressed ON cells irrespective of antinociceptive activity, but activated OFF cells in only those conditions where antinociception was present [35]. Thus, the available functional and physiological data indicate that antinociceptive activation of the vlPAG releases endogenous opioid peptides in the RVM that act through inhibition of ON cells for bicuculline-induced antinociception, and through activation of OFF cells for opioid antinociception.

Opioid Agonists in the vlPAG and Excitatory Amino Acid Antagonists in the RVM

Excitatory amino acid (EAA) receptors in the RVM have been implicated in antinociceptive processes given the actions of glutamate and NMDA [42, 75]. EAA antagonists in the RVM significantly reduce antinociception elicited by either electrical or opioid stimulation of the vlPAG [2, 91]. Our third series of studies [81] examined whether pretreatment of either competitive NMDA (AP-7), noncompetitive NMDA (MK-801) or kainate/AMPA (CNQX) EAA receptor antagonists into the RVM would alter morphine antinociception elicited from the vlPAG. The potent antinociception elicited by morphine in the vlPAG was significantly and dose-dependently reduced by RVM pretreatment with both competitive NMDA antagonism on the tail-flick (60%) and jump (86%) tests (fig. 3, top panels) and noncompetitive NMDA antagonism on the tail-flick (100%) and jump (100%) tests (fig. 3, middle panels). In contrast, RVM pretreatment with a kainate/AMPA antagonist failed to significantly alter morphine antinociception elicited from the vlPAG (fig. 3, lower panels). Microinjections of the NMDA antagonists failed to alter basal nociceptive thresholds in the RVM, and failed to alter morphine antinociception elicited from the vlPAG in misplaced medullary sites.

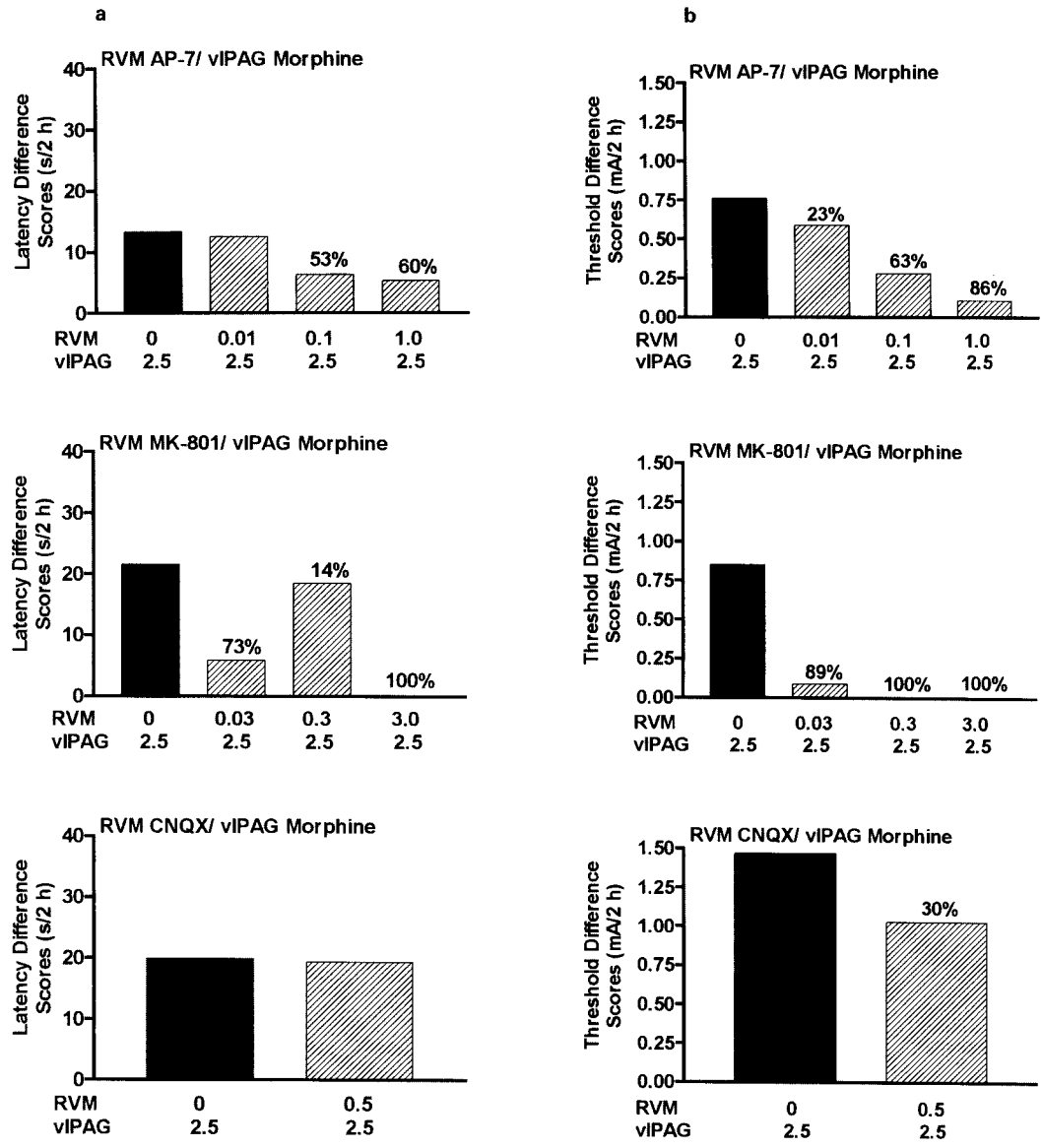

Fig. 3. Alterations in morphine antinociception on the tail-flick (**a**) and jump (**b**) tests elicited from the vlPAG following pretreatment with competitive (AP-7) and noncompetitive (MK-801) NMDA or kainate/AMPA (CNQX) EAA antagonists in the RVM.

Do these functional effects of NMDA antagonists in the RVM upon morphine antinociception in the vlPAG have any relationships with physiological responses of identified RVM neurons? EAA transmission appears to be responsible for ON-cell nociceptive activation in the RVM since iontophoretic application of the EAA antagonist, kynurenate, blocked this response [36], but EAA transmission appears to be completely unrelated to OFF-cell firing [33]. However, RVM administration of kynurenate prior to systemic morphine blocked the opioid-induced activation of OFF cells, and significantly reduced antinociception [34], thereby providing clear relationships between functional and physiological responses in the RVM by EAA agents in modulating supraspinal opioid antinociception.

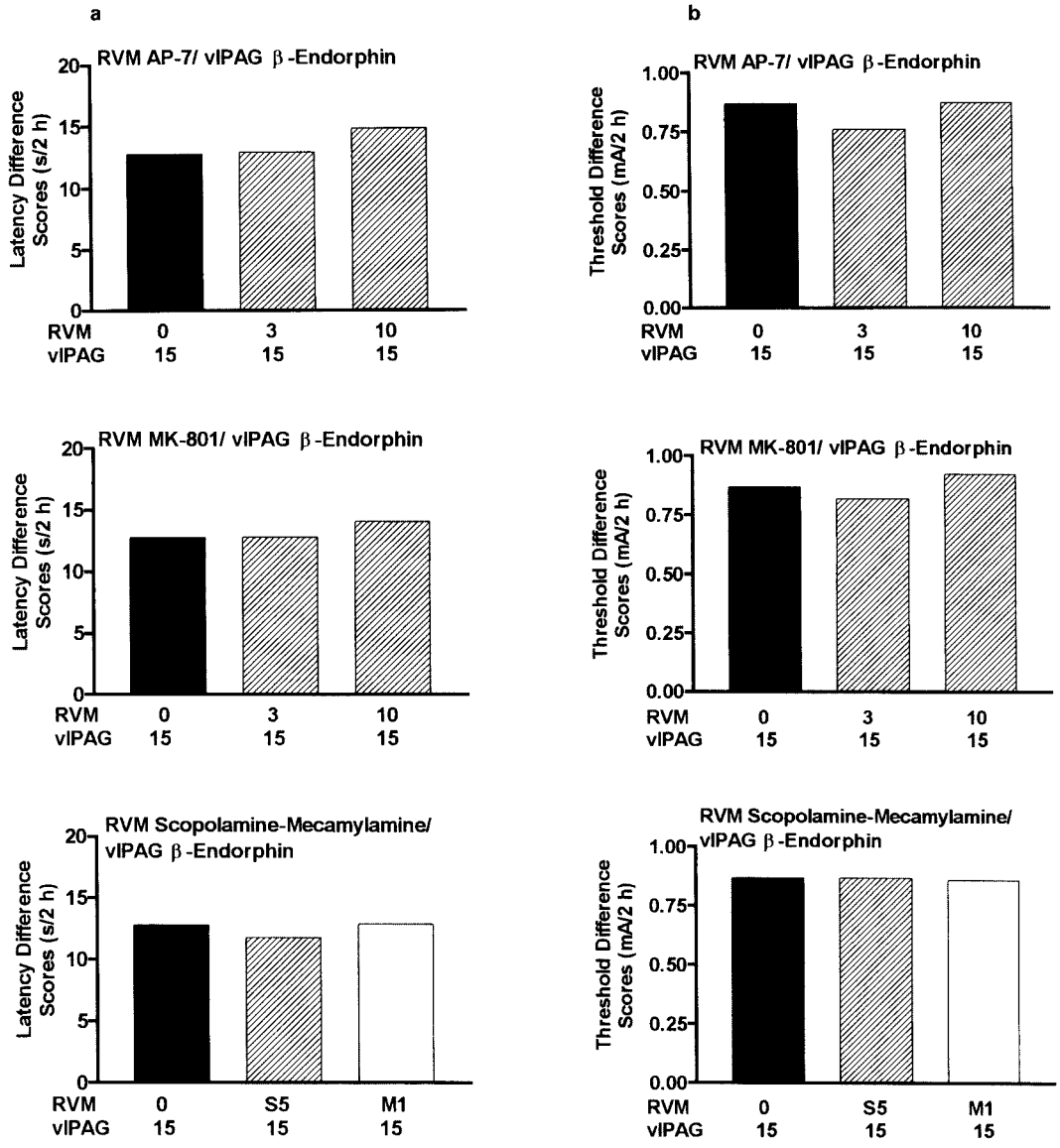

Fig. 4. Alterations in β-endorphin antinociception on the tail-flick (**a**) and jump (**b**) tests elicited from the vlPAG following pretreatment with competitive (AP-7) and noncompetitive (MK-801) NMDA antagonists or cholinergic antagonists (scopolamine-mecamylamine) in the RVM.

Morphine and the opioid peptide, β-endorphin, appear to utilize different mechanisms of action in producing opioid antinociception [see review 85] especially within the vlPAG. The two opioid drugs in the vlPAG display differential responses to barbiturate anesthesia [79], and are differentially mediated by spinal adrenergic, serotonergic and opioid receptor antagonists [55]. Separate subpopulations of μ-opioid receptors appear to mediate morphine and β-endorphin antinociception in the vlPAG given the nonparallel slopes of the dose inhibition curves induced by naltrexone and CTOP [54, 80]. Our laboratory [83] found agonist-induced specificity of RVM NMDA-induced mediation of antinociception elicited from the vlPAG in that β-endorphin antinociception elicited from the vlPAG failed to be altered by either competitive (fig. 4, upper panels) or noncompetitive (fig. 4, middle

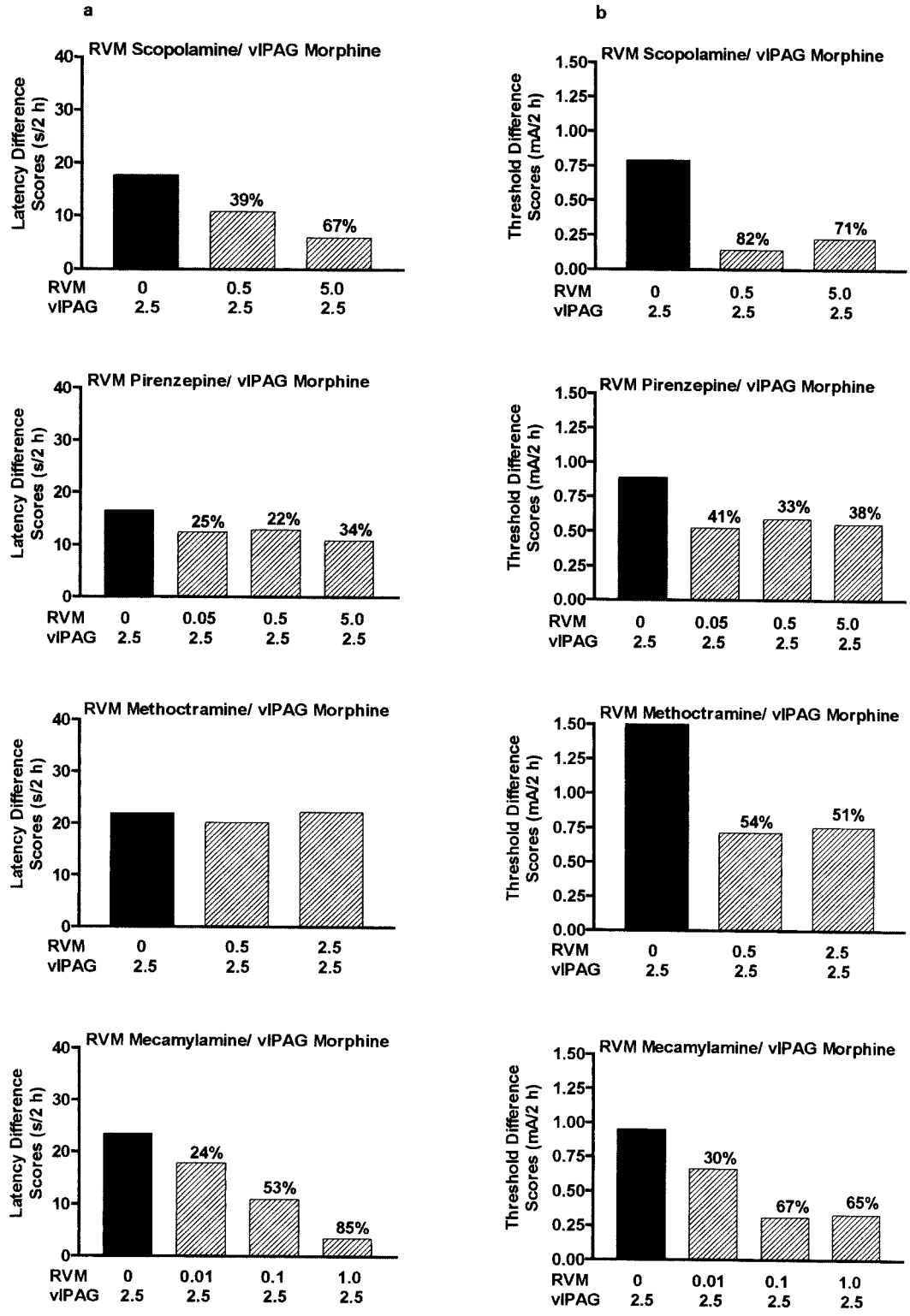

Fig. 5. Alterations in morphine antinociception on the tail-flick (**a**) and jump (**b**) tests elicited from the vlPAG following pretreatment with muscarinic (scopolamine), M_1 (pirenzepine), M_2 (methoctramine) or nicotinic (mecamylamine) cholinergic antagonists in the RVM.

panels) NMDA antagonists administered into the RVM at doses that were 100-fold higher than effective doses blocking morphine antinociception elicited from the vlPAG. Therefore, these data provide evidence for opioid agonist-induced specificity within the vlPAG in utilizing RVM circuitry in mediating antinociceptive responses.

Opioid Agonists in the vlPAG and Cholinergic Antagonists in the RVM

The RVM is a major site of antinociceptive action for cholinomimetic drugs [15, 41, 47]. Therefore, a fourth series of studies [82] examined whether pretreatment of either muscarinic (scopolamine), M_1 (pirenzepine), M_2 (methoctramine) or nicotinic (mecamylamine) cholinergic receptor subtype antagonists in the RVM would alter morphine antinociception elicited from the vlPAG. Morphine antinociception elicited by morphine in the vlPAG was significantly and dose-dependently reduced by RVM pretreatment with scopolamine on the tail-flick (67%) and jump (71%) tests (fig. 5, top panels). This antagonism was selective since scopolamine microinjections failed to alter basal nociceptive thresholds in the RVM, and failed to alter morphine antinociception elicited from the vlPAG in misplaced medullary sites. However, neither M_1 nor M_2 antagonism in the RVM altered morphine antinociception elicited from the vlPAG on the tail-flick test (fig. 5, left middle panels), and the reductions in morphine antinociception elicited from the vlPAG on the jump test were smaller following pirenzepine (8%) and methoctramine (54%) (fig. 5, right middle panels). These effects were also not site-specific since these antagonists were effective in medullary placements dorsal and lateral to the RVM. The significant reductions in morphine antinociception elicited from the vlPAG on the tail-flick (85%) and jump (67%) tests by RVM mecamylamine pretreatment (fig. 5, lower panels) were also not site-specific since dorsal and lateral medullary placements were also effective in reducing morphine antinociception. Our laboratory [83] also determined that these cholinergic antagonist effects were selective to the opioid antagonist employed since neither scopolamine nor mecamylamine pretreatment in the RVM significantly altered β-endorphin antinociception elicited from the vlPAG on either the tail-flick or jump tests (fig. 4, lower panels). Although these series of studies indicated that the integrity of morphine, but not β-endorphin antinociception elicited from the vlPAG is dependent upon serotonergic, opioid, NMDA, and to a less-specific extent, cholinergic receptor subtypes in the RVM, Urban and Smith [86, 87] have demonstrated that neurotensin in the RVM modulates morphine antinociception elicited from the vlPAG in an opposite manner. Thus, whereas neurotensin itself in the RVM significantly reduces morphine antinociception elicited from the vlPAG, neurotensin antagonists in the RVM significantly enhance this response.

Opioid Agonists in the Amygdala and Opioid Antagonists in the vlPAG

Opioid-opioid interactions between sites have thus been described between the vlPAG and RVM, between the vlPAG and habenula, and between the habenula and nucleus accumbens in which opioid agonists in one site are blocked by opioid antagonists in a second site [46, 49]. A fifth series of studies [61] examined the relationship between opioid antinociception elicited from the amygdala [39, 67] and opioid synapses in the vlPAG. As described in previous paradigms, rats were tested for morphine and β-endorphin antinociception on both the tail-flick and jump tests. These agonists produced very minimal antinociception as measured by the tail-flick test in awake, freely moving rats in these studies, although greater responsiveness to these antinociceptive effects were observed on the tail-flick test using anesthetized rats [38–40]. The marked antinociception elicited by morphine in the amygdala on the jump test was significantly reduced by general (naltrexone, 79%) and δ_2 (naltrindole isothiocyanate, 60%) opioid antagonists, but not by μ (β-FNA) opioid antagonists in the vlPAG (fig. 6, upper panels). Similarly, β-endorphin antinociception in the amygdala on the jump test was significantly reduced by general (93%) and δ_2 (79%), but not by μ-opioid antagonists in the vlPAG (fig. 6, lower panels). The κ_1-agonist, U50488H elicits antinociception following ventricular administration, but fails to produce antinociception following microinjection into either the vlPAG, locus coeruleus or RVM [13, 14, 71]. Our laboratory [60] also determined that the antinociception elicited by U50488H on the jump, but not the tail-flick test in the amygdala was significantly reduced by κ_1-antagonist pretreatment in the amygdala, and by either general, μ- or δ_2-antagonist pretreatment in the vlPAG. That an opioid synapse in the vlPAG is necessary for the full expression of opioid antinociception elicited from the amygdala was also demonstrated by the observation that antinociception elicited by the μ-selective agonist, DAMGO, in the basolateral amygdala was significantly reduced by general and μ-, but not

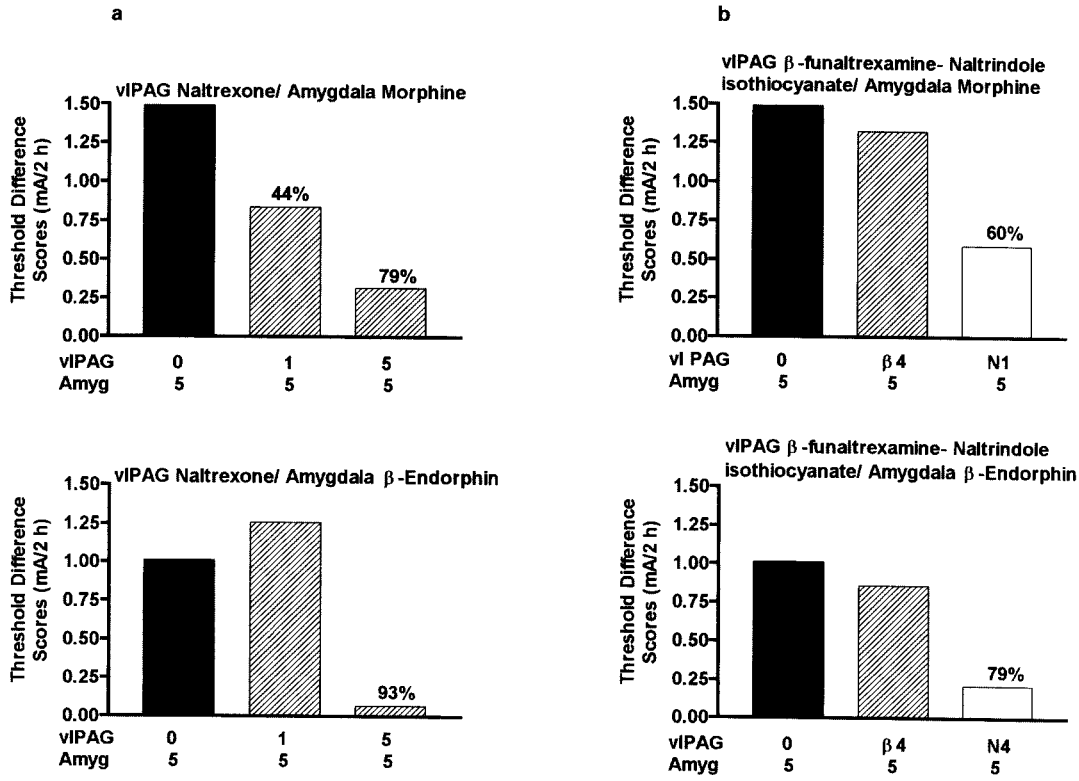

Fig. 6. Alterations in morphine or β-endorphin antinociception on the jump test elicited from the amygdala following pretreatment with general (naltrexone; **a**) or selective [β-funaltrexamine (μ)-naltrindole isothiocyanate (δ_2); **b**] opioid antagonists in the vlPAG.

by β-endorphin$_{1-27}$ in the vlPAG [84]. Further, lidocaine injections into either the vlPAG or the RVM blocked the expression of DAMGO antinociception elicited from the basolateral amygdala [40].

Supraspinal Synergy Studies between Sites

The presence of synergistic antinociceptive interactions has been used to assess functional relationships between spinal and supraspinal opioid systems [68, 69, 95]. Our laboratory examined whether synergistic antinociceptive interactions for morphine and selective opioid agonists occurred between pairs of supraspinal sites. We [70] first determined whether functional interactions occurred for the anatomical connections between the vlPAG and the RVM [1, 12, 89, 90], between the vlPAG and locus coeruleus [4, 17, 22, 66], and between the RVM and locus coeruleus [3, 19]. In this and the following paradigms, full dose-response curves for each agonist were ascertained on the nociceptive test in each site alone, and subsequently in pairs of sites in which one site would receive a fixed, subthreshold agonist dose, and the second site would receive a range of subthreshold doses. Thus, a dose of morphine that failed to increase latencies when administered into either the vlPAG or RVM alone produced a significant and marked antinociception following simultaneous administration (fig. 7a). This antinociceptive response was more marked for vlPAG-RVM interactions than for either locus coeruleus-RVM or vlPAG-locus coeruleus interactions. Indeed, administration of a fixed subthreshold dose of morphine into the vlPAG produced a 10-fold leftward shift in the morphine dose-response curve in the RVM, while the subthreshold dose of morphine in the RVM produced a 3-fold leftward shift in the morphine dose-reponse curve in the vlPAG. We [71] then determined the opioid receptor subtypes involved in vlPAG-RVM interactions, and found that simultaneous administration of subthreshold doses of the μ-selective agonist, DAMGO, into both sites produced a

synergistic interaction as did simultaneous administration of subthreshold doses of the δ_2-selective agonist, *D*-Ala^2, Glu^4-deltorphin (fig. 7b). Indeed, if a subthreshold dose of a μ-agonist was applied to one site, and a subthreshold dose of a δ_2-agonist was applied to the second site, synergistic antinociceptive interactions also occurred, implying that these interactions involve pathways rather than receptors per se. In contrast, simultaneous administration of either κ_1- or δ_1-opioid agonists failed to either elicit antinociception in single sites or produce synergistic interactions when applied simultaneously to pairs of sites.

Our laboratory [59] finally examined whether synergistic antinociceptive interactions also occurred between the amygdala and vlPAG for morphine and β-endorphin. Simultaneous administration of subthreshold doses of morphine into both sites produced a synergistic interaction as did simultaneous administration of subthreshold doses of β-endorphin (fig. 7c). This antinociceptive interaction persisted when subthreshold doses of β-endorphin in the amygdala were coadministered with subthreshold doses of morphine in the vlPAG. However, no interaction was observed following coadministration of subthreshold doses of morphine into the amygdala and β-endorphin into the vlPAG, presumably because β-endorphin is activating a different neurochemical circuit within the vlPAG than morphine [54, 55, 80–83].

Conclusions

These data conclusively establish that functional relationships exist between supraspinal sites mediating opioid antinociception. The well-established connection between the vlPAG and RVM appears to be dependent upon multiple neurochemical systems within the RVM for its full expression in antinociceptive responses. Moreover, functional relationships have been established between the vlPAG and locus coeruleus, between the locus coeruleus and RVM, and between the amygdala and

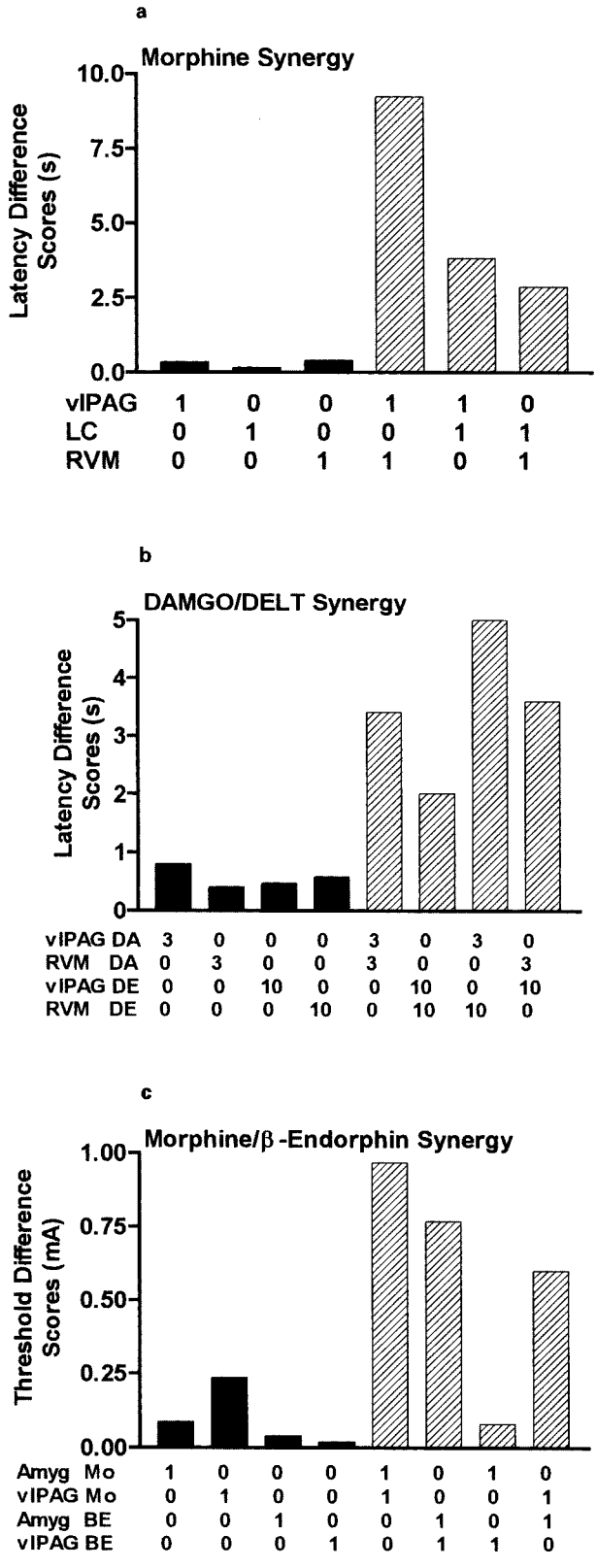

Fig. 7. a Synergistic antinociceptive interactions are observed for subthreshold doses of morphine administered into pairs of supraspinal sites, including the vlPAG, locus ceruleus (LC) and RVM. **b** Synergistic antinociceptive interactions are observed for subthreshold doses of selective μ (DAMGO: DA)- and δ_2 (deltorphin: DE)-opioid agonists between the vlPAG and RVM. **c** Synergistic antinociceptive interactions are observed for subthreshold doses of morphine (Mo) and β-endorphin (BE) between the amygdala and vlPAG.

vlPAG. It appears that regionalinteractions may best explain antinociceptive responsiveness, and that alterations within one part of this complex system may produce subtle changes in the responsiveness of the entire system to different types of nociceptive input.

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