

## NORADRENERGIC TERMINAL EXCITABILITY: EFFECTS OF OPIOIDS

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The local infusion of morphine or D-Ala<sup>2</sup>,Met<sup>5</sup>-enkephalinamide into the frontal cortical terminal fields of noradrenergic neurons of the nucleus locus coeruleus resulted in a decrease in the excitability of the axon terminal regions to direct electrical stimulation. These effects were concentration dependent and could be blocked or partially reversed by the local infusion of naloxone. Some evidence was obtained for a differential antagonizing effect of naloxone upon the effects of morphine and D-Ala<sup>2</sup>,Met<sup>5</sup>-enkephalinamide. These results are discussed with respect to an effect of opioids on the polarization and/or ionic conductance of the terminal fields of locus coeruleus neurons, and to the possible regulation of neurotransmitter release by presynaptic opiate receptors.

The rat nucleus locus coeruleus, composed principally of noradrenergic neurons, has been shown to be rich in opiate receptors [14]. The systemic and microiontophoretic administration of morphine and opiate peptides have been shown to inhibit the spontaneous activity of locus coeruleus neurons [2, 6, 7, 16, 18] which in the guinea pig locus coeruleus (in vitro) is thought to be due to a hyperpolarization of the soma-dendritic membranes [13].

A number of biochemical studies have presented evidence for the existence of presynaptic opiate receptors located on nerve terminals of central noradrenergic neurons [1, 9, 17]. Activation of presynaptic opiate receptors by morphine or opioid peptides has been reported to cause a decrease in stimulus-evoked norepinephrine release from central noradrenergic nerve terminals, an effect similar to that seen upon stimulation of presynaptic  $\alpha$ -adrenergic receptors on these neurons [8, 17].

We have previously obtained evidence suggesting that stimulation of presynaptic autoreceptors on the terminal membranes of dopaminergic and noradrenergic neurons leads to a decrease in the excitability of their terminal fields to direct

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electrical stimulation, whereas autoreceptor blockade leads to an increase in the terminal excitability of these catecholaminergic nerve cells [5, 11], effects attributable to changes in the polarization and/or ionic conductance of the terminal membrane. On the basis of these previous results, the present experiments were designed to elucidate the effects of morphine and  $\text{D-Ala}^2, \text{Met}^5$ -enkephalinamide (ENK), a stable synthetic opiate peptide analogue, on the terminal excitability of locus coeruleus neurons.

Forty-six male Sprague-Dawley rats (250–450 g) were anesthetized with urethane (1.3 g/kg, i.p.) and prepared for extracellular recording from locus coeruleus as previously described [10, 11]. Terminal excitability was determined, as previously detailed [5, 11], by measuring the current necessary to antidromically activate locus coeruleus neurons from frontal cortex before and after the infusion of various concentrations of morphine, ENK, and naloxone into the site of stimulation at a rate of 0.0625 ml/min for 5 min (for details of methods, see ref. 11). Responses were characterized as antidromic in nature provided that they exhibited collision extinction with spontaneous action potentials occurring within a period of time before and after the stimulus delivery corresponding to the antidromic latency plus the refractory period. Morphine at various concentrations ranging from 1 to 40  $\mu\text{M}$  was infused via 32-gauge cannulae directly into the frontal cortical terminal fields of 27 locus coeruleus neurons. Morphine produced a significant decrease in excitability for all doses combined ( $t = 4.90$ ,  $df = 26$ ,  $P < 0.001$ ). Terminal excitability decreased in 21 cells, increased in 2 cells, and was unchanged in the remaining 4 cells. Changes in terminal excitability were observed within 1–2 min from the start of the infusion. As shown in Table I, the effect of morphine appeared to be concentration-dependent.

Fig. 1A demonstrates a typical dose-related change in terminal excitability for one locus coeruleus neuron following local infusion of morphine. Local infusion of 40  $\mu\text{M}$  morphine led to a decrease in terminal excitability and a uniform shift to the right in the current–response curve. A further decrease in terminal excitability was seen following a subsequent infusion of 40  $\mu\text{M}$  morphine.

ENK was infused into the frontal cortex in 16 cases at a concentration of 10  $\mu\text{M}$  and, like morphine, decreased terminal excitability ( $t = 2.62$ ,  $df = 15$ ,  $P < 0.02$ ). Terminal excitability decreased ( $\bar{X} \pm \text{S.E.M.} = 40.4 \pm 12.1\%$ ) in 11 of 16 cells following local infusion, increased ( $16.7 \pm 3.4\%$ ) in 2 cells, and remained unchanged in the remaining 3 cells (Table I). A typical dose-related decrease in terminal excitability of a locus coeruleus neuron following successive local infusions of 10  $\mu\text{M}$  ENK is shown in Fig. 1B.

Local infusion of an opiate antagonist, naloxone, was examined in 20 locus coeruleus neurons. The majority of the cells (11/20) did not show any change in terminal excitability following local infusion of 10  $\mu\text{M}$  naloxone alone ( $t = 1.16$ ,  $df = 19$ ,  $P = 0.261$ ), whereas at this concentration the antagonist led to increased terminal excitability ( $22.5 \pm 2.5\%$ ) in 7 neurons and decreased terminal excitability in the remaining 2 neurons ( $18.7 \pm 7.1\%$ ) (Table I).

TABLE I  
EFFECTS OF LOCAL INFUSION OF MORPHINE, ENK AND NALOXONE INTO FRONTAL CORTEX UPON TERMINAL EXCITABILITY OF LOCUS COERULEUS NEURONS

Excitability	Morphine						ENK 10 $\mu$ M	Naloxone 10 $\mu$ M
	1 $\mu$ M	4 $\mu$ M	10 $\mu$ M	40 $\mu$ M	Pretreatment with 10 $\mu$ M naloxone 10 $\mu$ M	ENK 10 $\mu$ M		
Decreased	2 <sup>a</sup> (25.0 $\pm$ 12.5) <sup>b</sup>	3 (30.7 $\pm$ 14.8)	10 (45.8 $\pm$ 6.2)	6 (57.5 $\pm$ 21.0)	6 (7.1 $\pm$ 1.1)	11 (40.4 $\pm$ 12.1)	2 (22.5 $\pm$ 2.5)	
Increased	0	0	2 (16.2 $\pm$ 6.2)	0	1 (18.7)	2 (16.7 $\pm$ 3.4)	7 (18.7 $\pm$ 7.1)	
No effect	0	1	2	1	2	3	11	
Total	2	4	14	7	9	16	20	

<sup>a</sup>Numbers indicate the number of cells responding in the indicated manner.

<sup>b</sup>The numbers in parentheses indicate mean change in threshold current as per cent of control values  $\pm$  S.E.M. Threshold is defined as the minimum stimulating current sufficient to evoke an antidromic response on 100% of the non-collision trials. From 20–100 stimulus presentations were made at each current intensity.

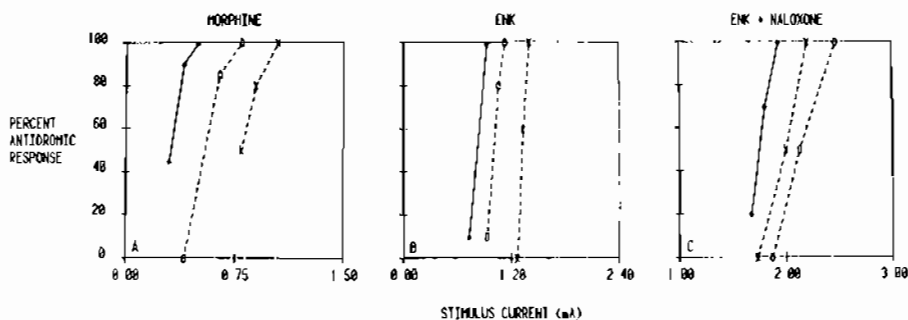


Fig. 1. Dose-related changes in terminal excitability of locus coeruleus neurons following local infusion of opioid agonists into the frontal cortex. Solid curves in all graphs indicate control excitability curves. A: infusion of  $40 \mu\text{M}$  morphine produced a decrease in terminal excitability, as revealed by the increase in stimulus currents necessary to evoke antidromic responses (0—0). A second infusion of  $40 \mu\text{M}$  morphine was followed by a further reduction in the terminal excitability ( $\times$ — $\times$ ). B: infusion of  $10 \mu\text{M}$  ENK produced decreased terminal excitability (0—0). A second infusion at the same concentration led to a further decrease in the terminal excitability ( $\times$ — $\times$ ). C: the local infusion of  $10 \mu\text{M}$  naloxone causes a partial reversal of the decreased terminal excitability produced by a prior infusion of  $10 \mu\text{M}$  ENK into the same cortical site. The current necessary to evoke 100% antidromic response was increased by 28% following  $10 \mu\text{M}$  ENK (0—0). Infusion of  $10 \mu\text{M}$  naloxone into the same site led to 50% recovery towards the control level of terminal excitability ( $\times$ — $\times$ ).

Changes in terminal excitability produced by the opioids were correlated with alterations in antidromic response latency; decreased terminal excitability was often accompanied by an increase in the latency (0.5–1.0 msec) and in latency variability, whereas increased terminal excitability was usually associated with a decrease in the latency and in the latency variability of the antidromic response. Local infusions of opioids and naloxone were not accompanied by changes in firing rate.

In order to examine the antagonizing effect of naloxone on the opioid agonists, either morphine or ENK was infused into the frontal cortex, terminal excitability was re-assessed and naloxone was administered either intravenously (0.25–0.5 mg/kg) or by local infusion ( $10 \mu\text{M}$ ) into the same site in the frontal cortex.

A typical antagonizing effect of naloxone upon an opiate agonist is demonstrated in Fig. 1C. The decreased terminal excitability produced by a prior infusion of  $10 \mu\text{M}$  ENK was partially reversed by local infusion of  $10 \mu\text{M}$  naloxone. Both local infusion ( $n = 3$ ) and intravenous injection ( $n = 3$ ) of naloxone led to an attenuation of the effect of prior ENK infusion.

In contrast to its effects on ENK infusion, the antagonizing effect of naloxone on the decreased terminal excitability following morphine infusion was more difficult to demonstrate. In 10 cases of a prior infusion of morphine, subsequent naloxone infusion produced a small antagonizing effect in 3 cases, and had no effect in 7 cases.

To further examine the interactions between morphine and naloxone with respect

to terminal excitability, naloxone was infused into the frontal cortex prior to infusion of morphine in 9 cases. The antagonizing effects of naloxone on morphine were apparent in the following results: (1) when the frontal cortex was pretreated with  $10 \mu\text{M}$  naloxone, the onset latency of the decrease in terminal excitability following local infusion of  $10 \mu\text{M}$  morphine into the same site (3–4 min) was longer than that seen when morphine was infused without pretreatment with naloxone (1–2 min); (2) in sharp contrast to the cases without naloxone pretreatment, local infusion of morphine ( $10 \mu\text{M}$ ) with naloxone pretreatment resulted in a significantly smaller ( $t = 2.65$ ,  $df = 21$ ,  $P < 0.015$ ) decrease in terminal excitability (Table I); (3) immediately after the cessation of morphine infusion, the decreased terminal excitability produced by morphine quickly returned to control levels, in contrast to the prolonged excitability changes ( $> 15$  min) seen in the absence of naloxone.

Because Pepper and Henderson demonstrated that opiate agonists produce a hyperpolarization associated with an increase in membrane conductance in locus coeruleus neurons in vitro [13], it seems possible that the decreased terminal excitability produced by the opiate agonists is also due to a hyperpolarization and/or conductance increase in the terminal membranes of locus coeruleus neurons. As demonstrated by biochemical studies, activation of presynaptic opiate receptors, as well as presynaptic  $\alpha$ -adrenergic receptors, leads to inhibition of stimulation-evoked norepinephrine release from these terminals [1, 8, 17]. The mechanism of this inhibition is as yet unclear, but has been suggested to involve a decrease in voltage-dependent calcium current [4], possibly due to a reduction in the electrotonic propagation of the nerve impulse into the synaptic bouton [11, 12]. A hyperpolarization of the terminal membrane would favor this type of inhibition. Therefore, it is possible that a change in polarization or conductance of the terminal membrane may underlie the process common to the regulation of norepinephrine release by presynaptic  $\alpha$ -adrenergic and opiate receptors.

Although the terminal excitability of the majority of locus coeruleus neurons was not affected by naloxone infusion, in a number of cases (35%) the opiate antagonist produced an increase in terminal excitability. It is thus conceivable that these results reflect a small tonic inhibition of the noradrenergic axon terminals by an endogenous opiate.

Naloxone locally infused before, but not after morphine infusion, could partially block the effects of a subsequent morphine infusion. In contrast the decreased terminal excitability produced by ENK infusion was partially but consistently antagonized by a subsequent administration, either locally or systemically, of naloxone. The precise reasons for this difference are not clear from the present experiments, but might include differential binding affinities of opiates, opioid peptides and antagonists [3], an alteration in the opiate receptor conformation subsequent to morphine binding [15], or the presence of multiple opiate receptors on the nerve terminals.

These results provide further supporting evidence for the existence of functional

presynaptic receptors located on the terminals of locus coeruleus neurons, and support the view that inhibition of norepinephrine release from the terminals by opiate agonists could be effected not only by an inhibition of spontaneous activity, but also by a presynaptic receptor-mediated local inhibitory action of opioids on noradrenergic synaptic terminals.

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