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Opiate and α_2-Adrenoceptor Responses of Rat Amygdaloid Neurons: Co-localization and Interactions during Withdrawal[1]

JONATHAN E. FREEDMAN[2] AND GEORGE K. AGHAJANIAN

Departments of Psychiatry and Pharmacology, Yale University School of Medicine and Ribicoff Research Facilities, New Haven, Connecticut 06508

Abstract

Interactions between neuronal responses mediated by opiate receptors and by α_2-adrenoceptors were characterized in the amygdala. Extracellular single-unit recordings and microiontophoresis were performed using five-barrel microelectrodes in chloral hydrate-anesthetized rats. A subpopulation of amygdaloid cells displayed inhibitory responses to morphine or D-Ala,D-Leu-enkephalin; antagonist studies suggested that both μ- and δ-opiate receptor subtypes were present. The same neurons displayed inhibitory responses to norepinephrine or clonidine mediated by α_2-adrenoceptors.

Responses mediated by opiate receptors and by α_2-adrenoceptors were highly co-localized to the same subpopulation of amygdaloid neurons. Such cells responded to microiontophoresis of either morphine or clonidine, whereas other cells in the amygdala generally showed neither response. Responsive cells were characterized by a distinctive, triphasic waveform and a high sensitivity to glutamate. These cells were largely restricted to the nucleus centralis and the posterior portion of the nucleus medialis. Cells outside of this group showed suppressant responses to norepinephrine which appeared not to be mediated by α_2-adrenoceptors.

After chronic morphine treatment, application of opioid antagonists elicited a withdrawal response, consisting of an increase in firing rate. Clonidine reversed the withdrawal response of these cells. The amygdala may be one of the regions of the nervous system in which clonidine acts to reduce symptoms of opiate withdrawal.

The α_2-adrenergic agonist clonidine is clinically useful in reducing the symptoms of opiate withdrawal (Gold et al., 1978). Many attempts to characterize the location in the brain in which the interactions between clonidine and opiates might occur have focused on the norepinephrine-containing neurons of the locus ceruleus (Aghajanian, 1978; Redmond and Huang, 1982; Andrade et al., 1983). These brainstem cells display direct inhibitory responses mediated both by opiate receptors and by α_2-adrenoceptors; after chronic morphine treatment their firing rates increase in response to the opiate antagonist naloxone—an opiate withdrawal response—and clonidine can reverse this response (Aghajanian, 1978). However, 6-hydroxydopamine lesions of the locus ceruleus or the dorsal noradrenergic bundle have no effect on the ability of clonidine to reduce certain behavioral signs of opiate withdrawal in rats, thus calling into question the idea that the locus ceruleus could be the only site of action of clonidine in relieving withdrawal symptoms (Britton et al., 1984).

The amygdala is a forebrain region known to play a major role in mediating a number of the behavioral signs of opiate withdrawal (Lagowska et al., 1978; Calvino et al., 1979). Several lines of evidence suggest that opiate-α_2 interactions might be possible in the amygdala. Autoradiographic studies have shown that the amygdala is rich in opiate receptor-binding sites (Goodman et al., 1980; Quirion et al., 1983; Moskowitz and Goodman, 1984), as well as in α_2-receptor-binding sites (Young and Kuhar, 1980; Unnerstall et al., 1984). Lesion studies have demonstrated that some of the [^{3}H] clonidine-binding sites in the amygdala are postsynaptic (U'Prichard et al., 1980) and, thus, would have been spared in the lesion studies of Britton et al. (1984). In behavioral studies, intra-amygdaloid injection of opiate agonists impairs retention of passive avoidance conditioning in rats, whereas injection of naloxone enhances retention (Gallagher and Kapp, 1981a); the α_1/α_2-antagonist phentolamine has the same effect as naloxone (Gallagher and Kapp, 1981b). In single-unit recording studies, inhibitory responses of rat amygdaloid neurons to norepinephrine were not antagonized by the α_1-antagonist WB-4101 or the β-antagonist sotalol (Wang and Aghajanian, 1980).

Because the amygdala seems to be a possible location for clonidine to exert actions on opiate withdrawal, we were prompted to examine amygdaloid cells for possible responses mediated by opiate and α_2-receptors. We have surveyed amygdaloid neurons of chloral hydrate-anesthetized rats using extracellular recording and microiontophoresis and now describe a distinctive subpopulation of cells in the central and medial nuclei to which inhibitory responses mediated by opiate receptors and by α_2-adrenoceptors are co-localized.

Materials and Methods

Drugs. Drugs were obtained from the following sources: morphine sulfate, Merck, Sharp and Dohme, Inc.; naloxone hydrochloride, Endo Laboratories; ICI 174864 (*N,N*-bisallyl-Tyr-(α-aminoisobutyric acid)$_2$-Phe-Leu-OH, which we converted to the sodium salt), Imperial Chemical Industries; clonidine hydrochloride, Boehringer Ingelheim, Ltd.; idazoxan hydrochloride, Reckitt and Colman, Ltd.; prazosin hydrochloride, Pfizer, Inc.; L-norepinephrine D-bitartrate, Regis Chemical Co.; D-Ala^2,D-Leu^5-enkephalin acetate, propranolol hydrochloride, L-phenylephrine hydrochloride, and monosodium L-glutamate, Sigma Chemical Co.

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[2] To whom correspondence should be addressed, at Department of Psychiatry, Yale University School of Medicine, 34 Park Street, New Haven, CT 06508.

Single-unit recording and microiontophoresis. Male Sprague-Dawley rats, 240 to 300 gm, were anesthetized with chloral hydrate (400 mg/kg, i.p.), with additional anesthetic given intravenously as needed. Extracellular recordings were made *in vivo* using five-barrel microelectrodes. The recording apparatus has been described previously (Wang and Aghajanian, 1977).

For microiontophoresis, five-barrel glass microelectrodes were prepared from five-barrel Omega Dot capillaries (W-P Instruments) as previously described (Freedman and Aghajanian, 1984). The center barrel was filled with 2 M NaCl, 2% Pontamine sky blue, and was used as the recording channel. One side barrel was filled with 3 M NaCl and used for automatic current balancing. The three remaining side barrels were filled with drug solutions. One drug solution was always 10 mM glutamate in 0.9% NaCl, pH 8.0. The other two drug solutions were various combinations of the following: morphine, 50 mM, pH 4.5; D-Ala,D-Leu-enkephalin, 5 mM in 0.9% NaCl, pH 4.0; ICI 174864, 4 mM in 0.9% NaCl, pH 8.0; clonidine, 0.1 M (except where indicated) in 0.9% NaCl, pH 4.0; idazoxan, 10 mM in 0.9% NaCl, pH 4.0; norepinephrine, 0.1 M in 0.9% NaCl, pH 4.0; phenylephrine, 0.1 M in 0.9% NaCl, pH 4.0. Electrode impedances, measured in saline at 60 Hz, were typically 4.5 to 9.0 megohms in the recording channel, 5 to 15 megohms in the balance channel, and 20 to 100 megohms in the drug channels. Glutamate and ICI 174864 were ejected as anions, the other drugs as cations. Retaining currents of +10 nA for ICI 174864, −10 nA for morphine, D-Ala,D-Leu-enkephalin, and idazoxan, and −15 nA for clonidine, norepinephrine, and phenylephrine, were applied between periods of ejection.

To record in the amygdala, a 3-mm burr hole was placed with its center 4.5 to 6.5 mm anterior to the lambdoid suture and 2.5 to 4.8 mm lateral to midline, with amygdaloid cells generally found 6 to 9 mm below the surface of the cortex. Routine coordinates to search for responsive cells (see "Results") were 5.5 mm anterior and 4.3 mm lateral for the nucleus centralis, and 4.9 mm anterior and 3.0 mm lateral for the posterior nucleus medialis.

To obtain steady cell firing under chloral hydrate anesthesia, glutamate was microiontophoresed throughout the experiment (Wang and Aghajanian, 1980). An ejecting current of −5 nA was applied while searching for cells. Upon encountering a cell, a retaining current of +20 nA was applied, after which a dose response to glutamate was determined. The maximal firng rate of the cell was defined as either the rate of firing immediately before the cell went into apparent depolarization blockade (observed extracellularly as a diminution of amplitude and lengthening of duration of action potentials at high firing rate, followed abruptly by a reversible cessation of firing) or as the steady rate which could not be increased by quadrupling the ejecting current. The glutamate current was then reduced so as to elicit firing at 50% of the maximal rate, and this current was maintained for the duration of the experiment.

Cell firing rates per 10-sec interval were averaged for at least 1 min following test drug application and compared with average base line firing rates over at least 3 min preceding drug application; changes of not less than 10% were considered significant. All microiontophoretic applications of test drugs were made repeatedly to verify consistency of responses and to rule out random fluctuations in cell firing. Drugs for systemic administration were injected in 0.9% NaCl through a lateral tail vein. To avoid residual drug effects, no further cells were tested after an intravenous injection.

Histology. Recording sites were marked with a spot of Pontamine sky blue at the end of each experiment by passing 10 μA of negative current through the recording channel for 30 min. The animal was then perfused with 10% buffered formalin. Serial 50-μm frozen sections were cut and stained with cresyl violet and neutral red. All assignments of recording sites to a given nucleus were based upon the location of the dye spot.

Chronic morphine treatment. Morphine pellets, 75 mg (National Institute on Drug Abuse), were implanted subcutaneously under halothane anesthesia, 1 pellet daily for 5 days. This treatment is sufficient to induce prominent behavioral signs of opiate dependence (Blasig et al., 1973). Recordings were made 24 to 29 hr after the last pellet was implanted.

Results

Co-localization of responses to morphine and to clonidine. Cells were found in the amygdala which displayed inhibitory responses to microiontophoresis of either morphine or clonidine (Fig. 1*A*). Other amygdaloid cells responded to neither agonist (Fig. 1*B*). Table I shows the results of a series of experiments in which 57 histologically localized cells were tested with electrodes containing both drugs. With a small number of exceptions, cells responded either to both agonists or to neither. Furthermore, the responsive cells were not randomly distributed throughout the amygdala, but were largely restricted to the nucleus centralis and the nucleus medialis. As shown, most of the cells in these two nuclei responded to both morphine and clonidine, although a small number of cells failed to respond to one or both drugs.

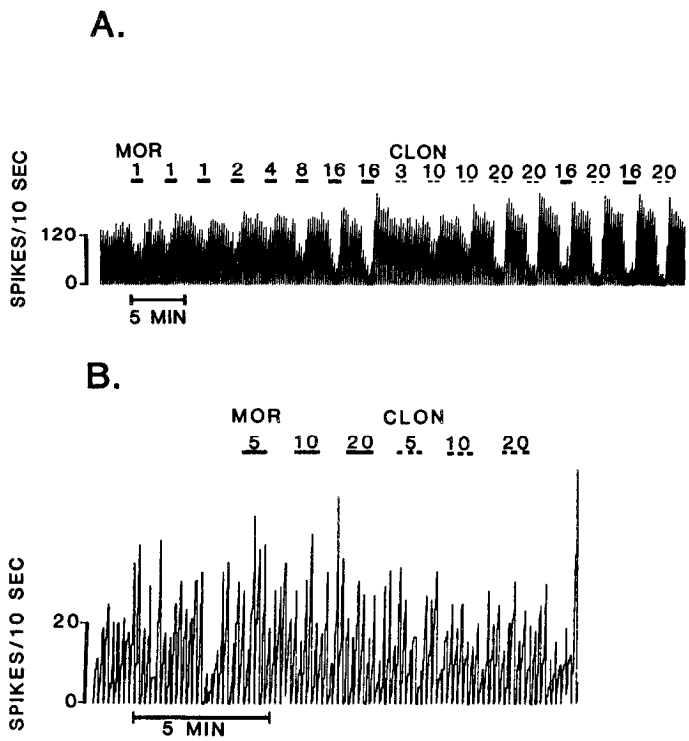

Figure 1. Examples of amygdaloid cells responsive and unresponsive to morphine (*MOR*) and clonidine (*CLON*). *A*, Record of a cell in the nucleus centralis which responded to both agonists. On this cell, each agonist elicited a full inhibition of firing; however, amplitudes of maximal responses to morphine and to clonidine were not identical on all cells. *B*, Record of a cell in the nucleus corticalis which responded to neither agonist. *Numbers* denote iontophoretic currents in nanoamperes.

TABLE I

Co-distribution of responses to morphine and to clonidine of amygdaloid neurons

Responses to each agonist were tested on 57 neurons recorded from using electrodes containing both agonists. Amygdaloid nuclei were defined according to the atlas of Konig and Klippel (1963).

Region	Cells Responding to				Total Cells Tested
	Morphine and Clonidine	Morphine Only	Clonidine Only	Neither	
Nucleus medialis	10	0	0	1	11
Nucleus centralis	6	0	1	1	8
Nucleus basalis (pars medialis)	2	0	0	11	13
Nucleus basalis (pars lateralis)	0	0	0	10	10
Nucleus corticalis	0	0	1	8	9
Nucleus lateralis	0	0	0	6	6
Total Amygdala	18	0	2	37	57

Figure 2 shows in greater detail the anatomical distribution of the cells listed in Table I. Responsive cells in the nucleus centralis were found diffusely throughout the nucleus. The few responsive cells in the nucleus basalis were found at its edge closest to the nucleus medialis. Responsive cells in the nucleus medialis were highly restricted to its posterior portion. They could be found at the extreme posterior aspect of the amygdala, extending anteriorly only as far as

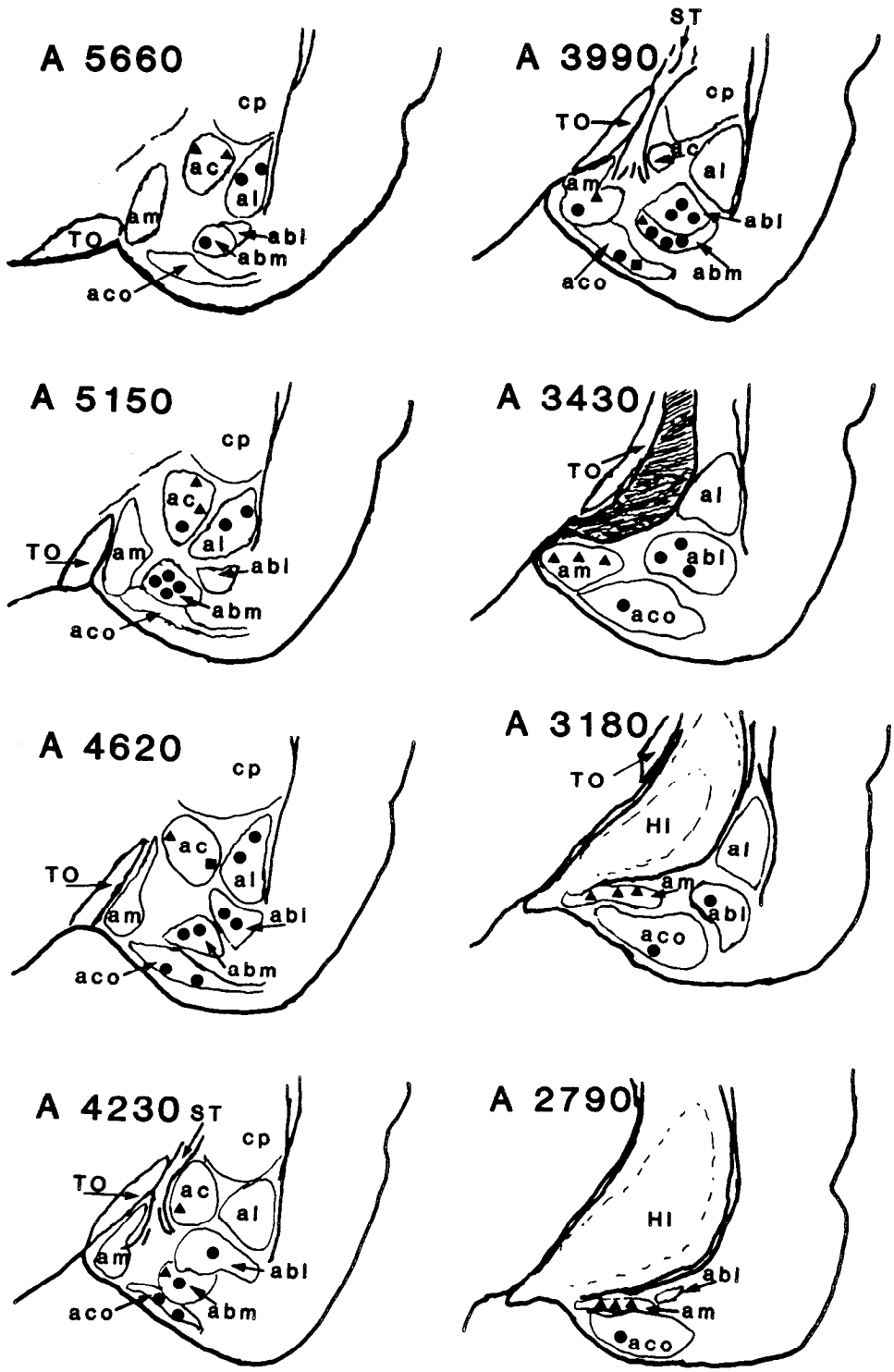

Figure 2. Anatomical distributions of the cells listed in Table I. ▲, cells responding to both morphine and clonidine; ■, cells responding to clonidine only; ●, cells responding to neither morphine nor clonidine. Stereotaxic levels and anatomical structures are labeled in coronal sections according to the atlas of Konig and Klippel (1963). Amygdaloid nuclei are: *abl*, nucleus basalis, pars lateralis; *abm*, nucleus basalis, pars medialis; *ac*, nucleus centralis; *aco*, nucleus corticalis; *al*, nucleus lateralis; *am*, nucleus medialis. Other structures are: *cp*, caudate-putamen; *HI*, hippocampus; *ST*, stria terminalis; *TO*, optic tract.

the level of the fiber bundle of the stria terminalis. Anterior to this level, no cellular potentials readily resolvable above background were found in the nucleus medialis, regardless of the glutamate current employed (+20 to −100 nA).

Pharmacology of opiate receptor responses. Responses to morphine of cells in the medial and central nuclei met the following pharmacologic criteria of being mediated by opiate receptors. Microiontophoresis of D-Ala,D-Leu-enkephalin as well as of morphine elicited a decrease in firing rate (Fig. 3*A*). The response to a given iontophoretic current of one agonist did not differ from the response to the same current of the other on a given cell, for seven cells tested with both agonists. Responses to agonists varied considerably from cell to cell, with variation in both the effective dose for a half-maximal response and in the amplitude and duration of the maximal response. Figure 3, *B* and *C*, shows records selected to illustrate variations in the amplitude of the response to iontophoretic morphine. Regardless of the amplitude of the maximal response, the iontophoretic current of morphine necessary to elicit a half-maximal response at 1 min varied from less than 1 nA to around 20 nA ($n = 38$ cells). Some cells (10 of 38) displayed a delayed increase in firing upon recovery from inhibition by morphine (Fig. 3*B*), possibly reflecting a disinhibitory effect mediated by adjacent cells. Systemi-

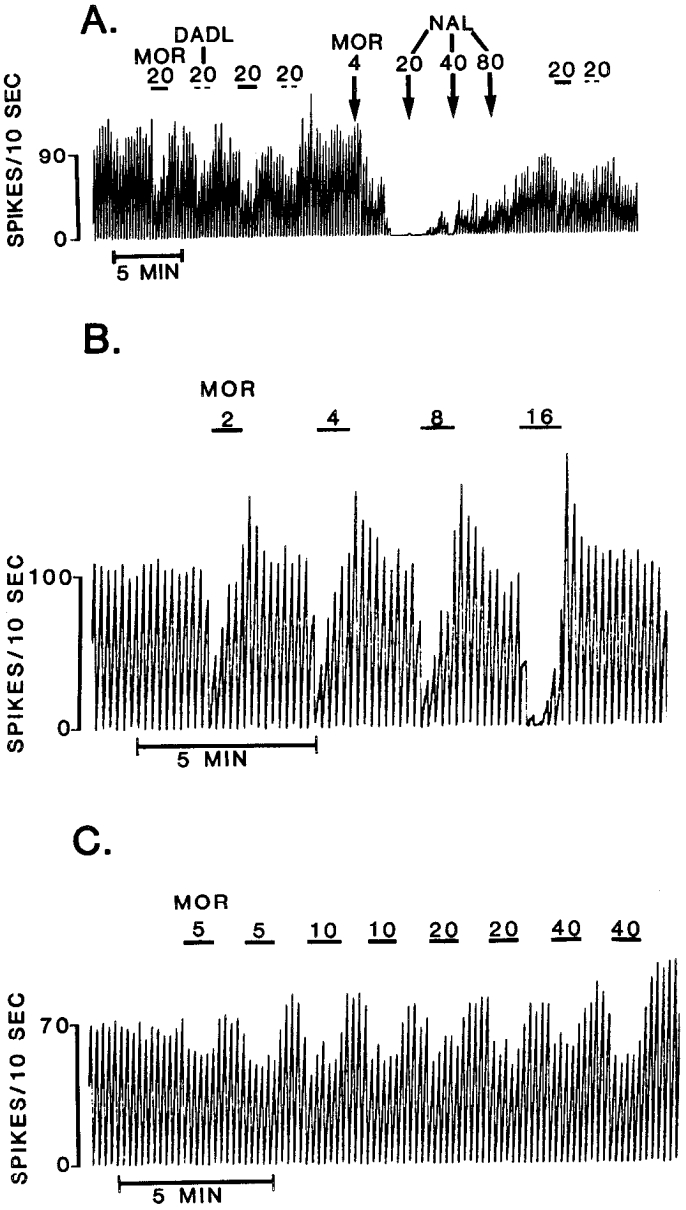

Figure 3. Responses of amygdaloid neurons mediated by opiate receptors. *A*, Responses to microiontophoresis of morphine (*MOR*, 20 nA) and D-Ala,D-Leu-enkephalin (*DADL*, 20 nA), and to systemic administration of morphine (4 mg/kg, i.v.), and antagonism of these responses by naloxone (*NAL*, 20 to 80 μg/kg, i.v., cumulative). *B* and *C*, Examples of responses to microiontophoresis of morphine, selected to illustrate the variability in the amplitude of the maximal response. *Numbers* in *B* and *C* denote iontophoretic currents in nanoamperes.

cally administered morphine also decreased cell firing (Fig. 3*A*), with effective doses for a 50% maximal response between 1 and 5 mg/kg, i.v. ($n = 5$ cells).

Naloxone at 50 to 500 μg/kg, i.v., gave partial to full antagonism of responses to morphine and D-Ala,D-Leu-enkephalin applied microiontophoretically, and to morphine administered intravenously (Fig. 3*A*). Of seven cells tested, all required doses of naloxone greater than 10 μg/kg, and three required doses greater than 100 μg/kg. Starting at doses around 100 μg/kg, a direct suppressant response to naloxone was observed (Fig. 3*A*). This is apparently the nonspecific local anesthetic-like effect which has been described by others for neurons in the cortex, hippocampus, thalamus, and caudate (Nicoll et al., 1977), since it entailed a diminution of action potential amplitude as well as of firing rate. When naloxone was applied microiontophoretically, this suppressant effect occurred at low ejecting currents, overlapping with the dose response for antagonism of responses to agonists, making iontophoretic results difficult to interpret.

The Leu-enkephalin analogue ICI 174864 has been reported to be a potent and selective antagonist at δ-subtype opiate receptors, with very little activity at μ-opiate receptors (Cotton et al., 1984; Dray and Nunan, 1984). Microiontophoresis of ICI 174864 at ejecting currents of −5 to −10 nA gave a full and slowly reversible antagonism of responses to morphine on some amygdaloid cells (Fig. 4*A*, $n = 4$ cells). On other cells ($n = 5$), the same currents of ICI 174864 had little effect (Fig. 4*B*). Both responses were encountered in the course of individual experiments, with the same electrode. Whereas naloxone typically displayed antagonist activity on all cells responding to morphine, albeit at high and variable doses, ICI 174864 tended to display all-or-none antagonism of morphine on a given cell. ICI 174864 also displayed a prolonged direct suppressant effect on cell firing (visible in Fig. 9*A*), but this most typically occurred at currents of −15 to −20 nA. It was not clear whether this was a local anesthetic-like effect or the partial agonist activity which has been described for this drug (Dray and Nunan, 1984). The α_2-adrenergic antagonist idazoxan (see below) did not antagonize responses to morphine (not shown, $n = 2$ cells).

Pharmacology of α_2-adrenoceptor responses. Responses to clonidine of cells in the medial and central nuclei met the following pharmacologic criteria of being mediated by α_2-adrenoceptors. Idazoxan (RX 781094), an α_1-antagonist with greater selectivity between central α_2- and α_1-receptors than piperoxane, yohimbine, or rauwolscine (Doxey et al., 1983; Freedman and Aghajanian, 1984), antagonized responses to clonidine, whether the drugs were applied microiontophoretically (Fig. 5*A*, $n = 6$ cells) or intravenously (Fig. 5*B*, $n = 6$ cells). Such an antagonism was obtained with a dose of idazoxan of 80 μg/kg, i.v., which is known to fully antagonize α_2 responses of locus ceruleus and dorsal raphe cells without antago-

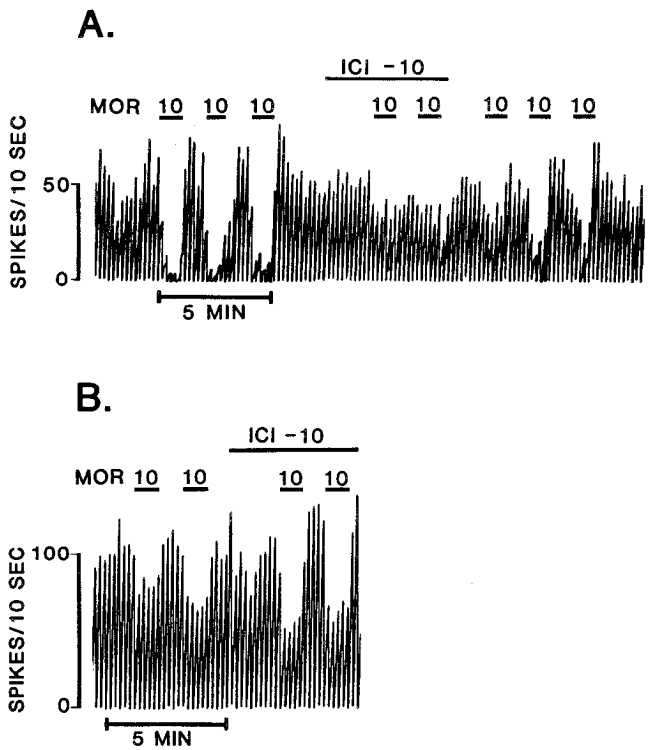

Figure 4. Variable antagonism of amygdaloid opiate receptor responses by ICI 174864 (*ICI*). *A*, Antagonism of a response to morphine (*MOR*). *B*, Failure to antagonize morphine on a different cell. *Numbers* denote iontophoretic currents in nanoamperes.

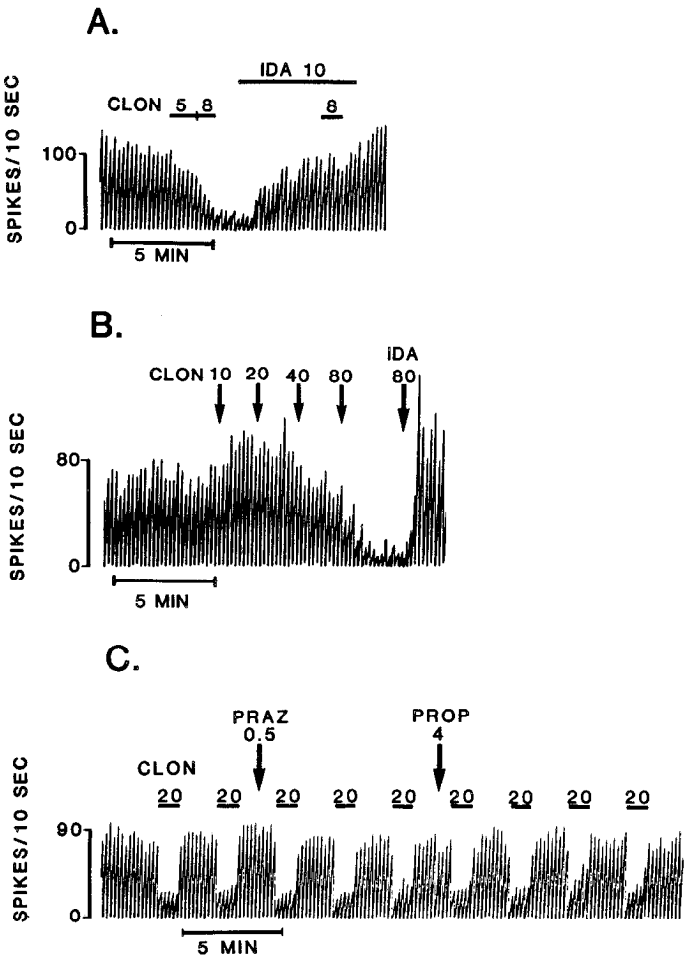

Figure 5. Responses of amygdaloid neurons mediated by α_2-adrenoceptors. *A*, Response to microiontophoretic clonidine (*CLON*) and its antagonism by microiontophoretic idazoxan (*IDA*). *Numbers* in *A* denote iontophoretic currents in nanoamperes. For *A* only, the concentration of clonidine in the electrode barrel was 10 mM, in 0.9% NaCl, pH 4.0. *B*, Response to systemically administered clonidine and idazoxan. *Numbers* in *B* denote cumulative intravenous doses in micrograms per kilogram. *C*, Failure of prazosin (*PRAZ*, 0.5 mg/kg, i.v.) or propranolol (*PROP*, 4 mg/kg, i.v.) to antagonize clonidine (20 nA).

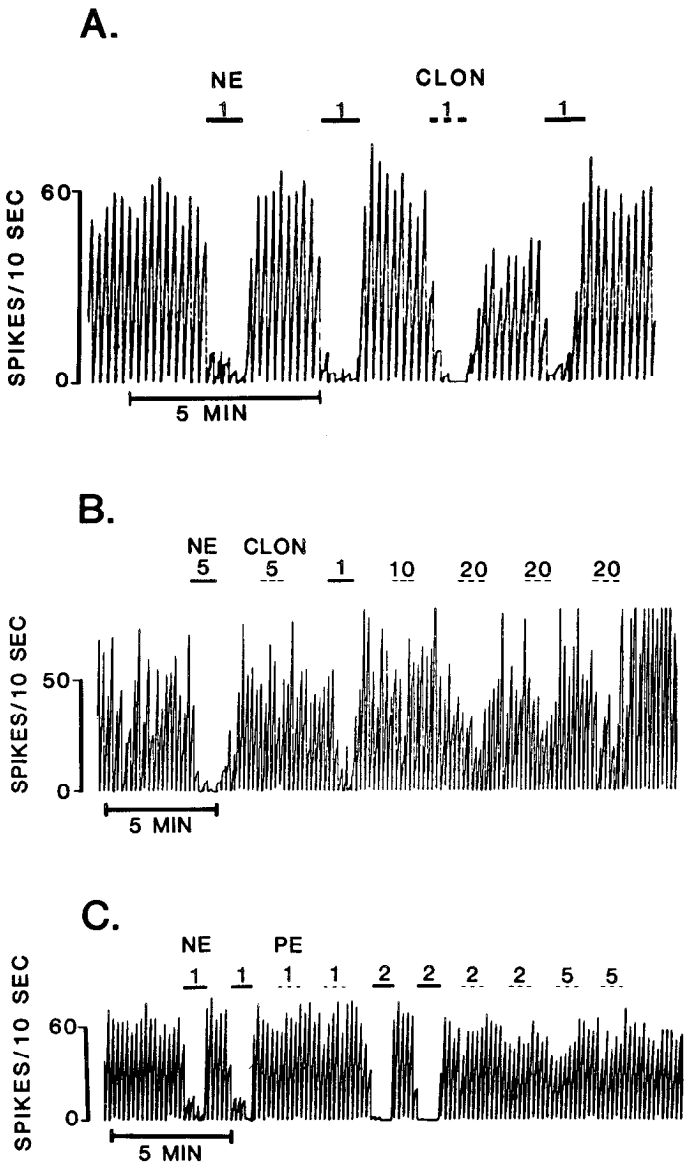

Figure 6. Responses of amygdaloid neurons to microiontophoresis of α-adrenoceptor agonists. *A* and *B*, Responses to norepinephrine (*NE*) and clonidine (*CLON*), selected to illustrate the variability of the potency of clonidine relative to norepinephrine. *C*, Comparison of phenylephrine (*PE*) with norepinephrine. *Numbers* denote iontophoretic currents in nanoamperes.

nizing α_1 responses (Freedman and Aghajanian, 1984). In contrast, the α_1-selective antagonist prazosin (0.5 mg/kg, i.v., $n = 3$ cells) and the β-antagonist propranolol (4 mg/kg, i.v., $n = 3$) were consistently ineffective at antagonizing responses to clonidine (Fig. 5*C*). Naloxone also did not antagonize responses to clonidine ($n = 8$ cells; see Fig. 9).

Cells responding to clonidine also responded to microiontophoresis of norepinephrine; clonidine generally required higher currents than did norepinephrine, with its ratio of potency relative to norepinephrine varying from cell to cell (Fig. 6, *A* and *B*). Norepinephrine consistently inhibited firing 90 to 100% at 1 min at currents of 0.5 to 2.0 nA ($n = 14$ cells). Of a total of 40 cells responding to microiontophoretic clonidine, 29 required currents above 2 nA to inhibit firing 90 to 100% (Fig. 6*B*), whereas 6 responded to currents similar to effective currents for norepinephrine (Fig. 6*A*), and 5 displayed large, prolonged responses to low iontophoretic doses of clonidine (Fig. 5*A*). The partially α_1-selective agonist phenylephrine (Cedarbaum and Aghajanian, 1977) was a weak agonist on these cells, showing only weak responses at currents at the high end of the dose range for clonidine (Fig. 6*C*, $n = 5$ cells).

Low systemic doses of clonidine frequently elicited increases in firing rates (Fig. 5*B*). Doses of 10 to 20 μg/kg, i.v., elicited a 15 to 29% increase in firing on three cells, had no effect on the firing of two cells, and elicited a 40% decrease in firing on one cell. Inhibitory responses on all six cells were obtained at doses of 40 to 100 μg/kg, i.v.

Properties of co-responsive neurons. Cells responding to both morphine and clonidine were generally distinguished from other cells in the amygdala by a characteristic extracellular waveform. Figure 7*A* shows the biphasic waveform of most amygdaloid neurons. Such potentials consisted of a prominent downward deflection followed by a smaller upward deflection, and had durations of 1.5 to 2.5 msec. Figure 7*B* shows the triphasic waveform encountered for cells responding to morphine and clonidine, consisting of an upward deflection with a small notch on its ascending phase, followed by a downward deflection, followed in turn by a second upward deflection usually, but not always, smaller in amplitude than the first upward deflection. These potentials had relatively long durations of 2.5 to 3.5 msec. Of the cells listed in Table I, all those in the nucleus

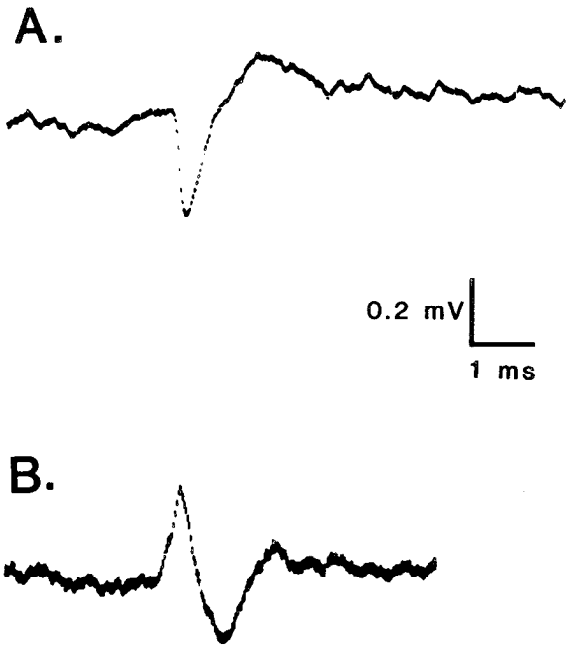

Figure 7. Single sweep traces of extracellular waveforms of amygdaloid neurons. *A*, Waveform of a cell in the nucleus basalis. *B*, Waveform of a cell in the nucleus medialis. Both these cells were recorded from in a single animal using the same electrode. Filters: 0.1 to 1.0 kHz bandpass.

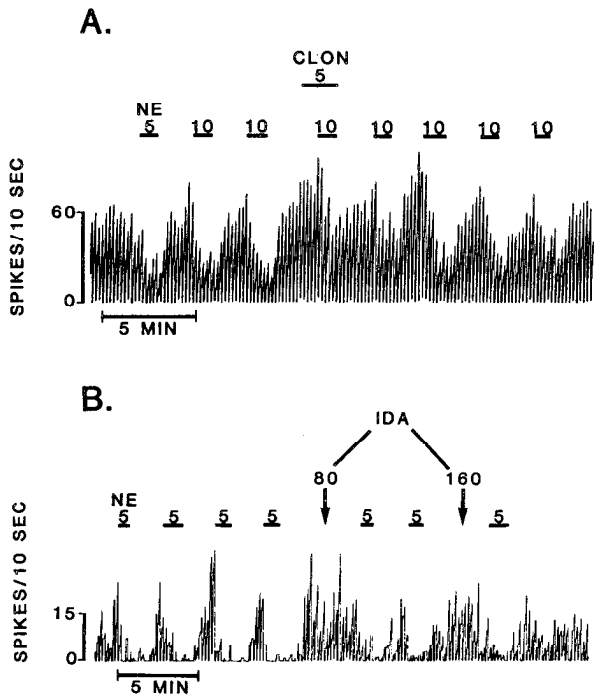

Figure 8. Responses to norepinephrine of amygdaloid cells lacking a direct inhibitory response to clonidine. *A*, Response of a cell in the nucleus basalis to microiontophoresis of norepinephrine (*NE*) and partial antagonism of this response by clonidine (*CLON*). *Numbers* in *A* denote iontophoretic currents in nanoamperes. *B*, Effect of idazoxan (*IDA*, 80 to 160 μg/kg, i.v. cumulative) on responses to norepinephrine (5 nA) of a cell in the nucleus corticalis.

centralis and nucleus medialis listed as responding to both morphine and clonidine displayed waveforms like that in Figure 7*B*, whereas all other cells listed in the table, including the two dually responsive cells in the nucleus basalis, had waveforms as in Figure 7*A*.

Responsive cells were also characterized by a high sensitivity to glutamate. They could usually be driven to fire by leakage of glutamate from the electrode under a small retaining current. Typically they were quiescent or only slowly firing at a current of +20 nA. They would fire at half-maximal rates at currents of +10 to −1 nA and would go into depolarization blockade at currents of −1 to −5 nA. Other cells rarely fired at positive glutamate currents and consistently required ejecting currents exceeding −10 nA to go into depolarization blockade. Although both cell types showed wide variability in the maximal firing rate elicited by glutamate, responsive cells generally displayed faster firing rates than did other cells, and generally were less prone to fluctuations in firing rate over time (cf. Fig. 1, *A* and *B*).

Norepinephrine responses of cells lacking a direct response to clonidine. Numerous amygdaloid cells displayed suppressant responses to microiontophoresis of norepinephrine, including many neurons outside of the cell groups where inhibitory responses to clonidine were found. Such responses to norepinephrine required higher currents (5 to 20 nA) to reduce firing 90 to 100% at 1 min (n = 8 cells) and typically displayed a latency of about 10 to 20 sec from the start of the ejecting current to the onset of the response. Clonidine at currents up to 30 nA failed to reduce the firing rates of these cells, but at currents of 1 to 10 nA it caused a slowly reversible antagonism of responses to norepinephrine (Fig. 8*A*, n = 5 cells). In contrast, clonidine failed to antagonize norepinephrine on cells where clonidine had agonist activity (Fig. 6*A*). Idazoxan was a weak and variable antagonist of responses antagonized by clonidine. At 80 μg/kg, i.v., it fully antagonized norepinephrine on one cell, partially antagonized it on one cell (Fig. 8*B*), and completely failed to antagonize it on three cells. Prazosin at 0.5 mg/kg, i.v. (n = 2 cells), and propranolol at 4 mg/kg, i.v. (n = 2 cells), did not antagonize these responses to norepinephrine.

Opiate withdrawal responses and clonidine. We examined interactions between responses mediated by opiate receptors and by α_2-adrenoceptors on seven triphasic-waveform amygdaloid neurons in six different rats during antagonist-induced opiate withdrawal after chronic morphine treatment. On every cell examined, application of opioid antagonists elicited a significant increase in firing rate; such increases were never observed in control animals (Fig. 9). ICI 174864 generally elicited somewhat larger responses than naloxone did. ICI 174864 at −5 nA (n = 7 cells) elicited responses ranging from a minimum increase of 25% (n = 1 cell) to a maximal increase of 100% followed by depolarization blockade (n = 2), with other responses intermediate. Naloxone at 50 μg/kg, i.v., (n = 5 cells), had no significant effect on the firing rate of one cell; for the other four cells naloxone-induced responses ranged from an increase of 12% (n = 1) to an increase of 100% with depolarization blockade (n = 1), with other responses intermediate, and with larger responses to naloxone on the cells with larger responses to ICI 174864. Clonidine at 100 μg/kg, i.v. (Fig. 9*B*, n = 5 cells), or 10 nA microiontophoretically (n = 2 cells, not shown) consistently reduced cell firing to below base line, cancelling out the antagonist-induced increase. In contrast, biphasic-waveform neurons in the nucleus basalis showed no response to ICI 174864 (−5 nA) after chronic morphine treatment (n = 3 cells), in the same animals where responses to ICI 174864 were observed on triphasic-waveform neurons.

Discussion

The major findings of this study are that responses of neurons in the rat amygdala mediated by opiate receptors and by α_2-adrenoceptors are, to a considerable extent, co-localized to a distinctive subpopulation of amygdaloid cells and that, although these inhibitory responses show some pharmacologic differences from responses in the locus ceruleus, they still display interactions resembling those found in the locus ceruleus. The cells to which the two responses were typically co-localized were distinguished by a characteristic

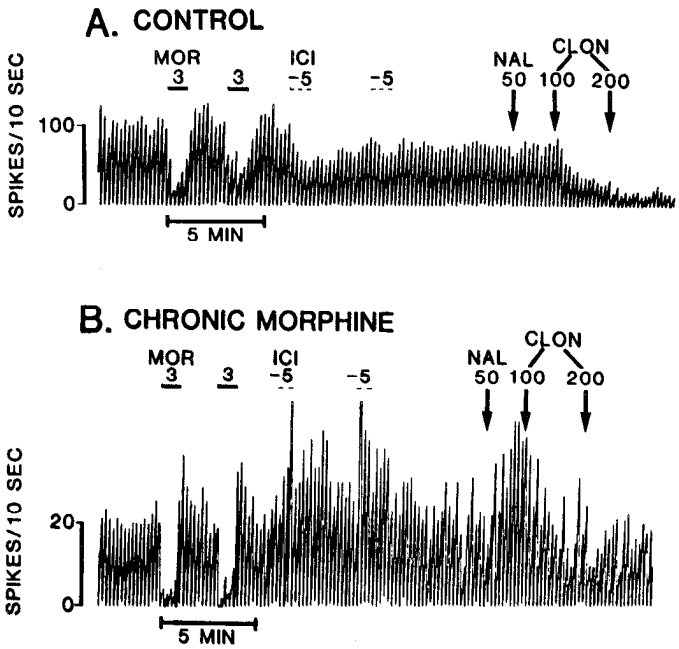

Figure 9. Opiate-α_2 interactions of amygdaloid cells in a control rat (*A*) and in a rat treated chronically with morphine (*B*). Each record shows responses to morphine (*MOR*, 3 nA), ICI 174864 (*ICI*, −5 nA), naloxone (*NAL*, 50 μg/kg, i.v.), and clonidine (*CLON*, 100 to 200 μg/kg, i.v., cumulative).

extracellular waveform and by a high sensitivity to glutamate. They were largely restricted to the nucleus centralis and to the posterior portion of the nucleus medialis. Further studies will be needed to elucidate the morphology of these cells, the synaptic connections that they make and receive, and the neurotransmitters that they contain. It seems clear that opiate and α_2 effects were mediated by distinct receptors, since idazoxan did not antagonize morphine and naloxone did not antagonize clonidine.

In order to be able to measure inhibitory responses in chloral hydrate-anesthetized rats, we have driven the firing of these cells by microiontophoresis of glutamate (Wang and Aghajanian, 1980). This method offers some advantages over the unanesthetized cerveau isolé preparation (Wang and Aghajanian, 1977). It elicits a fairly steady and rapid non-zero base line for measurement of inhibitory responses and does not require disruption of ascending axons synapsing onto the cells being studied. It also permits study of a larger population of amygdaloid cells, by not restricting study to spontaneously active cells. For instance, virtually all cells in the nucleus centralis, presumably including those which responded to morphine and clonidine in this study, are quiescent in the cerveau isolé preparation (R. Y. Wang and G. K. Aghajanian, unpublished observation). However, a disadvantatage of this approach is that depolarization of the cells by glutamate is likely to shift dose response curves for agonists. Thus, effective doses for inhibition of firing in this study may be greater than they would have been in an unanesthetized preparation. Glutamate may also have exerted modulatory effects by altering the release of endogenous neurotransmitters from adjacent cells onto the cell recorded.

Our antagonist data indicate strongly that the inhibitory effects of clonidine we observed were mediated by α_2-adrenoceptors; however, these responses differed in some ways from responses to clonidine in the brainstem. Iontophoretic clonidine (at 10 mM in 0.9% NaCl inside the electrode barrel) consistently elicits much larger, longer-lasting responses than does norepinephrine (at 0.1 M) in the locus ceruleus (Cedarbaum and Aghajanian, 1977). In the amygdala, however, we found clonidine (at 0.1 M) to act generally only at somewhat higher iontophoretic currents than norepinephrine, by an amount that varied from cell to cell. The use of glutamate may explain the difference in the absolute potency of clonidine between amygdala and locus ceruleus.

The difference in *relative* potency of clonidine to norepinephrine may be due to differences between α_2-receptors mediating responses in the amygdala and those in the locus ceruleus, with clonidine less effective at amygdaloid receptors. Alternatively, there may have been secondary actions of norepinephrine at receptors other than α_2-receptors. For instance, we have described suppressant responses of amygdaloid cells to norepinephrine which were antagonized by clonidine (Fig. 8). Although these responses might reflect an atypical form of α_2-receptor, we feel that the complete lack of clonidine agonist activity as well as the relative ineffectiveness of idazoxan as an antagonist argues instead that these responses are not mediated by α_2-adrenoceptors at all.

At doses of 10 to 20 μg/kg, i.v., which are known to consistently give full inhibition of firing of locus ceruleus neurons (Svensson et al., 1975), clonidine elicited increases rather than decreases in firing of amygdaloid cells (Fig. 5*B*), an effect which was never seen when clonidine was applied locally by microiontophoresis. The simplest interpretation of this increase in firing is that low doses of clonidine disinhibited amygdaloid cells by removing a tonic inhibitory noradrenergic input. This could occur if low doses of clonidine shut down the firing of neurons in the brainstem without directly acting on amygdaloid cells, either because the amygdaloid cells were activated by glutamate or because of lower potency of clonidine in the amygdala than at noradrenergic autoreceptors. A similar disinhibitory pattern has been described for postsynaptic cells in serotonergic systems after systemic administration of lysergic acid diethylamide (LSD) (Haigler and Aghajanian, 1974), and in dopaminergic systems after systemic administration of apomorphine (Skirboll et al., 1979).

An attractive interpretation of these results is that amygdaloid α_2-receptors are, at least in part, postsynaptic. This would imply that receptors in the amygdala would have been spared by lesions which failed to affect the ability of clonidine to reduce signs of opiate withdrawal (Britton et al., 1984), and would be consistent with radioligand binding data indicating that some of the α_2-binding sites in the amygdala are postsynaptic (U'Prichard et al., 1980). Although our ability to elicit responses in the amygdala by microiontophoretic application of agonists indicates that the responses are being mediated locally within the amygdala, we cannot rigorously distinguish between direct actions on the cells being recorded from, and actions on cells or cell processes adjacent to the cell recorded and synapsing directly or indirectly onto it. The rapid onset of responses to agonists and the disinhibitory effects of low systemic doses of clonidine provide only circumstantial evidence for direct postsynaptic effects of microiontophoretic agonists. Strictly speaking, we are describing localization of *responses* mediated by receptors, rather than localization of the receptors themselves.

Opiate receptor responses in the amygdala also differed from responses in the locus ceruleus. Receptors in the locus ceruleus are known to be of the μ-subtype (Williams and North, 1984). They are readily antagonized by naloxone at around 10 μg/kg, i.v. (Korf et al., 1974), whereas the δ-selective antagonist ICI 174864 is a weak antagonist (Williams and North, 1984). In the amygdala, naloxone proved significantly less potent, and ICI 174864 was highly effective as an antagonist on some cells and relatively inactive on others. ICI 174864 is known to act at nanomolar concentrations at δ-receptors in the mouse vas deferens (Cotton et al., 1984); micromolar concentrations are required at μ-receptors in the locus ceruleus (Williams and North, 1984). Although we cannot know the molar concentration of ICI 174864 in the tissue after iontophoresis, its differing effects on different cells suggest that it was present at a δ-selective concentration. Radioligand autoradiographic studies have shown rat amygdala to be rich in both δ- (Goodman et al., 1980) and μ- (Quirion et al., 1983) opiate-binding sites. It therefore seems likely that the responses that we measured were mediated in part by δ-receptors (those that were readily antagonized by ICI 174864), and in part by μ-receptors (those where ICI 174864 was inactive). Such a conclusion will require confirmation from rank-order potency studies of

opioid agonists. We found that similar iontophoretic currents were effective for morphine and for D-Ala,D-Leu-enkephalin; such comparisons are uninformative in terms of the relative potencies of these two compounds because of their differing iontophoretic mobilities, and *in vitro* studies may be needed to establish this information.

The anatomical distribution that we found for co-responsive cells is in fairly good agreement with autoradiographic data. The α_2-binding sites are most enriched in the nucleus centralis, nucleus medialis, and nucleus basalis pars medialis (Young and Kuhar, 1980). There is also a fairly high density of binding sites throughout the rest of the amygdala (Unnerstall et al., 1984); however, some of this binding may be associated with the site responsible for the effect of clonidine shown in Figure 8*A*. The μ- and δ-opioid-binding sites are intermixed in the mouse amygdala, with μ sites enriched in the nucleus centralis and nucleus medialis, and δ sites more uniformly distributed (Moskowitz and Goodman, 1984).

This anatomical distribution also agrees with functional data. The ability of microinjection of naloxone into the amygdala to elicit certain behavioral signs of opiate withdrawal in dependent rats is markedly localized to the nucleus centralis (Calvino et al., 1979). Localization, as well, to the nucleus medialis was not reported by those authors; however, their injection sites in that nucleus were all anterior to the cell group we have described. The ability of intra-amygdaloid injection of opiates to impair retention of passive avoidance conditioning is also localized to the nucleus centralis (Gallagher and Kapp, 1981a).

We have shown that, in rats treated chronically with morphine, these amygdaloid cells displayed opiate withdrawal responses, consisting of an increase in firing rate, in response to application of opioid antagonists. As in the locus ceruleus, it is likely that these reponses result from the abrupt antagonism of tissue morphine, elevating firing over a base line firing rate in the presence of morphine which has been altered as a result of tolerance (Andrade et al., 1983). In the amygdala, any such changes in base line firing would be obscured by the anesthetic and by glutamate. We speculate that in an unanesthetized animal the amygdaloid cells would be quiescent or slowly firing but would fire rapidly upon induction of opiate withdrawal. The ability of ICI 174864 as well as naloxone to elicit these responses suggests a role for δ-receptors in addition to μ-receptors in this response. We have not been able to document tolerance to morphine in these cells. There was no detectable decrease in sensitivity to acute morphine after chronic morphine treatment, for the number of cells tested, due to the heterogeneity of potency of responses to morphine, even in control animals. As in the locus ceruleus (Aghajanian, 1978), clonidine reversed the antagonist-induced opiate withdrawal response.

Lesions of noradrenergic neurons do not reduce the effectiveness of clonidine in reducing some behavioral signs of opiate withdrawal (Britton et al., 1984). Since such lesions would spare the amygdala, and since the amygdala plays a major role in mediating some opiate withdrawal behaviors (Lagowska et al., 1978; Calvino et al., 1979), it appears possible that the amygdala could be a site of action of clonidine in relieving withdrawal. However, it seems unlikely that such interactions should be restricted to only one region of the nervous system. In the forebrain, there is also functional evidence for opiate-α_2 interactions in the bed nucleus of the stria terminalis (Matsui and Yamamoto, 1984) which, interestingly, sends synaptic connections to, and receives synaptic connections from, the central and medial nuclei of the amygdala (Conrad and Pfaff, 1976; Weller and Smith, 1982). There is also considerable evidence for opiate-α_2 interactions in the spinal cord (Franz et al., 1982; Yasuoka and Yaksh, 1983). Thus, the amygdala is only one of a number of regions in the nervous system in which clonidine might act to lessen the symptoms of opiate withdrawal.

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