Opiate- and α_2 -Adrenoceptor-induced Hyperpolarizations of Locus Ceruleus Neurons in Brain Slices: Reversal by Cyclic Adenosine 3':5'-Monophosphate Analogues¹

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Abstract

The purpose of this study was to investigate the ionic and second messenger mechanisms underlying the hyperpolarizations induced by the selective α_2 -adrenoceptor agonist clonidine and the opiate agonist morphine in the locus ceruleus. Intracellular recordings were carried out in rat brain slices, and drugs at known concentrations were administered in the perfusate. The cyclic adenosine 3':5'-monophosphate (cAMP) analogues 8-bromo-cAMP and dibutyryl cAMP, while not altering basal activity, reversed the hyperpolarizations induced by clonidine or morphine. In contrast, administration of the parent compound adenosine failed to affect these responses. These results are consistent with previous biochemical studies suggesting that α_2 -adrenergic and opiate agonists might signal their actions by reducing intracellular cAMP levels.

Under manual voltage clamp, both clonidine and morphine elicited outward currents. The algebraic sum of the individual currents elicited by morphine and clonidine significantly exceeded the actual current elicited by their co-administration. This nonadditivity, as well as the observation that cAMP analogues reverse the morphine- and clonidine-induced hyperpolarizations, suggests that these compounds hyperpolarize locus ceruleus neurons through a shared ionic mechanism the activation of which might be signaled by a decrease in intracellular cAMP.

The locus ceruleus (LC) is a densely packed cell group located in the dorsal pons which contains approximately half of all noradrenergic neurons in the rat brain (Swanson and Hartman, 1975; Nygren and Olson, 1977). Previous extracellular studies *in vivo* have shown that LC neurons are inhibited by opiates (Korf et al., 1974; Bird and Kuhar, 1977) and α_2 -adrenoceptor agonists (Svensson et al., 1975; Cedarbaum and Aghajanian, 1977). The opiate-induced inhibition has recently been shown *in vitro* to result from a membrane hyperpolarization (Pepper and Henderson, 1980) mediated through an

increase in potassium conductance (Williams et al., 1982). Intracellular studies *in vivo* have suggested that the selective α_2 -adrenoceptor agonist clonidine (CLON) also hyperpolarizes LC neurons through an increase in potassium conductance (Aghajanian and Vander-Maelen, 1982). Similarities in the actions of opiate and α_2 -adrenoceptor agonists have also been reported in neuroblastoma X glioma hybrid cells, where both reduce intracellular levels of cAMP in a nonadditive manner suggesting a common biochemical mechanism (Sharma et al., 1975; Sabol and Nirenberg, 1979). Taken together, these findings raise the possibility that opiate and α_2 -adrenoceptor agonists, although acting via different receptors (Aghajanian, 1978; Franz et al., 1982), might hyperpolarize LC neurons through a common ionic mechanism the activation of which is signaled by decreases in cAMP levels. Such common mechanisms for the action of these agonists might be of functional significance in view of the reported ability of α_2 -adrenoceptor agonists to attenuate opiate withdrawal (Aghajanian, 1978; Gold et al., 1978). The present study was undertaken to characterize further the ionic mechanisms underlying the hyperpolarization induced by α_2 -adrenoceptor agonists in the LC, to examine whether cAMP analogues could modify the responses to opiate and α_2 -adrenergic agonists in this nucleus and to determine whether α_2 -adrenergic and opiate receptors in the LC share a common ionic mechanism to increase potassium conductance.

Materials and Methods

A total of 37 male albino rats (CAMP or Charles River) weighing 150 to 250 gm were used in these experiments. They were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed under 100% oxygen for at least 5 min prior to decapitation. The slicing procedure and general recording conditions were as previously described (Andrade et al., 1983; Andrade and Aghajanian, 1984). Briefly, pontine brain slices containing the LC were cut using a Sorvall Tissue Sectioner. They were incubated in a chamber modified from the design of Haas et al. (1979) and perfused with artificial cerebrospinal fluid (ACSF) of standard composition (in millimolar concentration: NaCl, 130; KCl, 5; NaH₂PO₄·H₂O, 1.25; NaHCO₃, 24; CaCl₂, 2.5; MgSO₄, 1.5; D-glucose, 15 mm; equilibrated with 5% CO₂, 95% O₂). The slices were kept at 33 to 34°C throughout the experiments. All recordings were obtained from the visually identified LC which appears under overhead illumination as a distinct dark area lateral to the central gray and IVth ventricle, immediately anterior to the descending Vllth nerve. Intracellular recordings were obtained with Kwik-fill (W-P Instruments) micropipettes filled with 3 M KCl (30 to 60 megohms); intracellular potentials were amplified using M707 (W-P Instruments) or Dagan 8100 (Dagan Corp.) amplifiers. Voltage drift was minimized by the use of silver/silver chloride leads for the electrode and ground connections and, in those experiments where reversal potentials (E_{revs}) were determined, the extracellular potential was ascertained immediately after drug administration. To prevent false readings of membrane potential during manual voltage clamp experiments, the bridge balance was continuously monitored intracellularly throughout these experiments. Recordings were considered acceptable if spontaneous activity did not exceed 4 spikes/sec, action potentials were at least of 60 mV amplitude, and the cells exhibited an apparent input

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resistance of at least 50 megohms when tested with a 0.2-nA hyperpolarizing pulse from about -55 mV. These were minimal requirements; cells with 80- to 100-mV action potentials and input resistances near 100 megohms were frequently encountered in these experiments. All cells reported here were impaled between 1 and 10 hr postdecapitation. All drugs were administered dissolved at known concentrations in the ACSF, and a drug-induced effect was considered at a plateau when there was no further change for at least 2 min. For experiments examining the possible summation between opiate and α_2 -adrenergic responses, a fresh slice was used for each experiment in order to prevent residual drug effects. Erevs were determined by establishing the current/voltage (IV) relationship of a given cell to a graded series of constant current pulses (200 msec) before and after drug administration (cf. Fig. 2). The Erev in this experiment corresponds to the voltage at which the two IV lines cross, as at that voltage the drug-induced current is zero. Although this method has the disadvantage of neglecting slow (>200 msec) voltage-dependent events, the more conventional approach, which involves short agonist administrations while holding the membrane potential at varying levels, was impractical because of the prolonged washout times required for morphine (MS) and CLON. Statistical comparisons used *t* tests based upon data obtained from chart paper records or oscilloscope photographs taken directly on line. Any holding current drift observed in the manual voltage clamp experiments was corrected by assuming constant drift through the whole clamping period. This procedure did not change the qualitative results from these cells. Clonidine (CLON) was obtained from Boehringer Ingelheim, morphine (MS) was from Merck, naloxone (NALOX) was from Endo Laboratories, and piperoxane (PIP) was from Rhone Poulenc.

Results

As expected from previous reports *in vivo* (Aghajanian and VanderMaelen, 1982) and *in vitro* (Andrade and Aghajanian, 1984), administration of CLON (200 nM to $1\text{ }\mu\text{M}$) hyperpolarized all LC neurons examined ($n = 8$ cells; Fig. 1A, bottom trace) and elicited a decrease in the amplitude of the electrotonic potential induced by hyperpolarizing current injections ($n = 8$ cells; Fig. 1A, top trace). This CLON-induced hyperpolarization exhibited an Erev of -90.5 ± 7.9 mV (mean $\pm$ SD, $n = 5$ cells) when determined in control ACSF containing 5 mM potassium (Fig. 2). Consistent with the suggestion that the CLON-induced hyperpolarization might be mediated by an increase in potassium conductance, its amplitude and Erev were both found to be dependent on the extracellular concentration of potassium. Thus, when the extracellular potassium concentration was doubled to 10 mM, the CLON-induced hyperpolarization was reduced (Fig. 2B), and its Erev shifted to -69.8 ± 4.1 mV (mean $\pm$ SD, $n = 5$ cells; Fig. 2). The magnitude of this shift (20.7 mV) approximated that predicted by the Nernst equation (18.3 mV/2-fold change in extracellular potassium at 34°C) for an effect mediated by a pure change in potassium conductance. As previously reported for opiates *in vivo* and *in vitro* (Korf et al., 1974; Bird and Kuhar, 1977; Pepper and Henderson, 1980; Williams et al., 1982), MS administration (1 to $5\text{ }\mu\text{M}$) also inhibited and hyperpolarized all LC neurons tested ($n = 6$; Fig. 1B). When the Erev for this hyperpolarization was determined in 5 mM external potassium and under conditions identical to those used for CLON, it was found to be -91.8 ± 1.8 mV (mean $\pm$ SD, $n = 5$ cells). This value was not found to be significantly different from that obtained for the Erev for CLON in 5 mM potassium ($p < 0.6$; Fig. 2).

Activation of α_2 -adrenergic and opiate receptors has been shown to elicit decreases in intracellular cAMP levels through an inhibition of adenylate cyclase (Sharma et al., 1975; Sabol and Nirenberg, 1979). Therefore, the possibility that decreases in cAMP might signal responses to α_2 -adrenergic and opiate agonists in the LC was examined by testing whether membrane-permeable cAMP analogues could reverse the effects of CLON and MS. The administration of CLON (200 to 500 nM, $n = 5$ cells) or MS (500 nM to $1\text{ }\mu\text{M}$, $n = 5$ cells) hyperpolarized all LC neurons tested and inhibited spontaneous activity when present ($n = 6$ cells; Fig. 3). The mean amplitudes for these hyperpolarizations were 13.5 ± 3.3 mV and 9.3 ± 1.5 mV (mean $\pm$ SD), respectively. When the membrane-permeable cAMP analogues dibutyryl cAMP (2 mM, $n = 2$ cells) or 8-bromo-cAMP (8-Br-cAMP) (500 μM to 2.0 mM, $n = 8$ cells) were

Figure 1. Effects of CLON and MS on input resistance (R_{input}) and membrane potential (V_m) in LC neurons. A, Top trace: CLON (800 nM) administration results in a decrease in R_{input} . R_{input} was determined according to Ohm's law ($R = V/I$) by injecting a hyperpolarizing current pulse of fixed amplitude and duration (0.2 nA for 200 msec in this case) and measuring the amplitude of the electrotonic potential elicited. The absolute amplitude of the electrotonic potential divided by the injected current (0.2 nA) is plotted in this trace. The decrease in R_{input} reflects the increase in membrane conductance induced by CLON as well as inward rectification by the cell membrane (Andrade and Aghajanian, 1984). Bottom trace: Administration of CLON results in a hyperpolarization and the cessation of spontaneous activity. This last effect is shown by the thinning of the V_m trace. The membrane potential of spontaneously active LC neurons varies during the firing cycle (Andrade and Aghajanian, 1984), producing a thickening of the trace as the recorder follows the slower components of the membrane potential fluctuations; spikes are not seen because of low-pass filtering by the recorder. Notice that the changes in membrane potential and membrane resistance underlying the firing cycle also elicit considerable variability in the R_{input} record since the hyperpolarizing pulses occur randomly throughout the interspike interval. The electrotonic potentials elicited by the hyperpolarizing pulses used to monitor R_{input} were "blanked" from the membrane potential trace by electronically holding the output to the recorder during the pulse at the level immediately preceding it. B, Top and bottom traces: Same as A except that MS ($1\text{ }\mu\text{M}$) was administered instead of CLON. Records A and B correspond to two different cells. The time bar applies to all traces.

administered to LC neurons in the presence of MS or CLON, they effectively reversed the opiate- and α_2 -adrenergic-induced hyperpolarizations in all cells tested: additionally, in five of the six cells which were spontaneously active in the absence of CLON or MS, the cAMP analogues were able to restore spontaneous firing (Fig. 3, A and B). The mean reversal induced by the cAMP analogues in the presence of MS was 10.2 ± 4.2 mV (mean $\pm$ SD) and in the presence of CLON was 12.3 ± 1.7 mV (mean $\pm$ SD). In addition to these effects on membrane potential, the administration of the cAMP analogues also reversed the decrease in the amplitude of the electrotonic potentials elicited by current injection which followed MS or CLON administration ($n = 5$ cells tested; Fig. 3A). Removal

Figure 2. Comparison of the reversal potentials for CLON and MS in the LC. **A**, Comparison of the reversal potentials (E_{rev} s) for CLON in ACSF containing 5 and 10 mM potassium and for MS in ACSF containing 5 mM potassium. The E_{rev} s for CLON (200 nM to 1 μ M) and MS (1 to 5 μ M) were indistinguishable in 5 mM potassium, but increasing the extracellular concentration of potassium to 10 mM resulted in a shift in the E_{rev} for CLON in the depolarizing direction. **B**, Examples of the raw data used to calculate the E_{rev} s shown in **A**. Hyperpolarizing pulses of increasing amplitude (right) were administered before and after drug administration to generate a pair of IV curves (left). Notice that, following CLON administration, the slope of the IV curve is reduced throughout the voltage range examined, indicating that the decrease in input resistance elicited by CLON did not simply result from anomalous rectification by the cell membrane. The E_{rev} corresponds to the potential at which the lines cross; i.e., the point where the net drug-induced current is zero. **Top record and plot**: Effect of CLON (1 μ M) in the presence of 5 mM potassium. **Middle record and plot**: Effect of CLON (1 μ M) in the presence of 10 mM potassium. **Bottom record and plot**: Effect of MS (1 μ M) in 5 mM potassium. Notice that, in the presence of 10 mM extracellular potassium, the hyperpolarization induced by CLON was reduced. ●, Pre-drug; ○, post-drug.

of the cAMP analogues from the agonist-containing perfusate led to a cessation of spontaneous activity (when present) and to a slowly developing hyperpolarization (Fig. 3, *A* and *B*). In contrast to the effects of the cAMP analogues, administration of adenosine (2 mM for 13 to 30 min) failed to produce any reversal of the CLON- or MS-induced hyperpolarizations ($n = 4$ cells, not shown). Administration of 8-Br-cAMP by itself (260 μ M to 2 mM for 15 to 30 min) elicited either no response or a slight increase (<1 spike/sec) in spontaneous activity ($n = 4$ cells; Fig. 3C). The effect of dibutyryl cAMP administered by itself was not tested.

The strong similarities in the E_{rev} s for CLON and MS as well as the reversal of the responses to both agonists by cAMP analogues raised the possibility that opiate and α_2 -adrenoceptor agonists might hyperpolarize LC neurons through a shared mechanism. Such an arrangement would predict, for a saturable mechanism, that an agonist would be much more effective when administered by itself than when administered in the presence of the other agonist. This situation would result in a nonadditivity of the individual effects of CLON and MS with respect to the effect elicited by both agonists administered together (Chad and Kerkut, 1981). Therefore, the additivity of the effects of CLON and MS in this cell group was investigated by using the manual voltage clamp method. This method entails the manual injection of current through the electrode to compensate for the slow drug-induced changes in potential. This procedure allows for direct quantification of drug responses while maintaining a steady membrane voltage. LC neurons were held at subthreshold holding potentials and agonist effects were quantified by measuring the current required to maintain the membrane voltage at that level following drug administration. The effects of near-

maximal concentrations of MS (1 to 2 μ M) or CLON (200 nM to 1 μ M) administered individually or in the presence of each other were determined by administration of MS, CLON, and their antagonists, NALOX and PIP in the following sequence: agonist 1 alone, agonist 1 and agonist 2 combined, agonist 2 plus antagonist of agonist 1, and finally both antagonists together (Fig. 4). In half of the experiments the first agonist administered (agonist 1) was CLON and in the other half MS was first. As illustrated in Figure 4, in all cases in which this procedure was completed ($n = 6$ cells), administration of the first agonist (either CLON or MS) resulted in a large increase in the holding current, reflecting the appearance of a large outward current. In contrast to the effect of the first agonist, administration of the second agonist resulted only in a small further increase in holding current, indicating that the second agonist had induced only a small additional outward current. These results were obtained independently of whether MS ($n = 3$ cells) or CLON ($n = 3$ cells) was administered first. Removal of the first agonist (which had originally induced a large outward current) in the presence of the second agonist produced only a small reduction in holding current (Fig. 4). In contrast, removal of the second agonist, which had previously induced only a small outward current, resulted in a large reduction in the holding current which brought it close to its initial level. Thus, the magnitude of the current induced by administration of the first agonist alone (e.g., CLON in Fig. 4) was much larger than the magnitude of the current elicited by its removal when in the presence of the second agonist. Similarly, the magnitude of the current elicited by the second agonist when in the presence of the first agonist was much smaller than the magnitude of the current elicited by its removal. Precise measurements of the drug-induced

Figure 3. Reversal of CLON- and MS-induced hyperpolarizations by 8-Br-cAMP. **A**, Bath administration of CLON (200 nM) hyperpolarizes an LC neuron and inhibits its spontaneous activity. Addition of 8-Br-cAMP in the continued presence of CLON reverses the hyperpolarization and elicits the resumption of spontaneous activity as shown by a thickening of the V_m trace. Removal of the 8-Br-cAMP while maintaining the CLON in the bath results in a slow hyperpolarization which can be reversed by PIP (15 μ M). Notice that the drug-induced changes in V_m are closely paralleled by changes in R_{input} . **B**, MS (1 μ M) administration hyperpolarizes an LC neuron and inhibits its spontaneous activity. Addition of 8-Br-cAMP to the MS-containing perfusate elicits a depolarization and the resumption of spontaneous activity. These effects are reversible, as removal of the cAMP analogue results in the cessation of spontaneous activity (indicated by the thinning of the line) which reflects the appearance of a small outward current. Re-administration of 8-Br-cAMP at this time can again elicit the resumption of spontaneous firing. R_{input} was not monitored in this cell. **C**, Administration of 8-Br-cAMP (2 mM) fails to depolarize or activate a spontaneously active LC neuron. Notice that in all experiments, including those illustrated here, drugs were administered continuously beginning at the arrow and at the concentrations specified in the legend. Records **A**, **B**, and **C** correspond to three different cells. The time bar applies to **A**, **B**, and **C**.

current was possible in five the above six cells and, in each of these, the algebraic sum of the individual agonist-induced currents was always larger than the current elicited by their co-administration. When the data from these five cells were pooled, the current induced by co-administration of both agonists was found to be significantly smaller than the sum of the currents elicited by the administration of MS and CLON individually (Table I).

Discussion

Previous studies in a number of systems including the mammalian CNS have shown that activation of opiate and α_2 -adrenergic receptors can result in an inhibition of adenylate cyclase and a decrease in cAMP levels (Sharma et al., 1975; Sabol and Nirenberg, 1979; Law et al., 1981; Limbird, 1983). Such actions might be of significance, for the signaling by these receptors as inactivation of the inhibitory guanine nucleotide-regulatory protein of adenylate cyclase (N_1) has been shown in peripheral tissues to block opiate and α_2 -adrenergic responses (Boyer et al., 1983; Tucker, 1984). Therefore, the possibility was considered in this study that decreases in cAMP might signal the effects of CLON and MS in the LC. Consistent with this possibility, administration of the membrane-permeable cAMP analogues, 8-Br-cAMP or dibutyryl cAMP, effectively reversed the hyperpolarization induced by MS or CLON. This reversal was slow in onset but subsided upon removal of the cAMP analogue, indicating that it did not result simply from tachyphylaxis to the effect of

the agonist. Of course it could be argued that the cAMP analogues could have simply depolarized these cells through a mechanism unrelated to that by which MS and CLON act. However, administration of 8-Br-cAMP by itself failed to significantly activate or depolarize LC neurons, suggesting that its action was to reverse the effect of the agonist rather than to produce an independent but opposite effect of its own. It could also be argued that these analogues might have activated adenosine receptors to reverse the effects of MS and CLON. However, adenosine administration failed to reverse the effects of CLON and MS, suggesting that adenosine receptor activation is probably not involved in these effects of dibutyryl cAMP and 8-Br-cAMP. Thus, the present findings are consistent with the concept that, in the LC, activation of α_2 -adrenergic and opiate receptors signal changes in ionic conductance across the membrane by decreasing intracellular cAMP levels.

Previous studies *in vitro* have shown that opiates hyperpolarize LC neurons through an increase in potassium conductance (Williams et al., 1982). If opiate and α_2 -adrenergic receptors signaled their actions through a common mechanism, it would be expected that the hyperpolarization induced by CLON should also be mediated through an increase in potassium conductance. Indeed, such a suggestion has previously been made (Aghajanian and Vandermaelen, 1982), based upon intracellular studies *in vivo*. In the present experiments, the E_{rev} for the CLON-induced hyperpolarization was found to shift with the extracellular potassium concentration, as would be expected for a change in potential mediated by a pure increase in potassium conductance. These observations, which are consistent with a recent report indicating that norepinephrine also hyperpolarizes LC neurons through an increase in potassium conductance (Egan et al., 1983), support the hypotheses that α_2 -adrenergic agonists, like opiates, hyperpolarize LC neurons through an increase in potassium conductance. Interestingly, when the E_{rev} s for CLON and MS were determined under identical conditions, they were found to be indistinguishable. Thus, as would be expected for a common signaling mechanism, CLON and MS both appear to open potassium channels of similar ionic selectivity and possessing a similar distribution in these neurons.

The effects of MS and CLON were found to exhibit a significant nonadditive behavior in the LC. Thus, the outward currents elicited by the administration of CLON or MS were markedly reduced when one of these agonists was administered in the presence of the other, and this reduction resulted in a nonadditivity of their individual effects when compared to their effect when administered together. Such observations suggest that opiate and α_2 -adrenergic receptors in the LC act through a shared mechanism to increase potassium conductance. These results resemble those seen for serotonin and dopamine in the viscerosubganglia of *Helix aspersa* where they both stimulate adenylate cyclase and elicit nonadditive decreases in potassium conductance (Deterre et al., 1982). They contrast, however, with those seen in neurons of the subesophageal ganglion of this snail, where serotonin and acetylcholine depolarize these cells through the same ionic mechanism but where both neurotransmitters exhibit perfect additivity in their responses (Chad and Kerkut, 1981). Conceivably, the nonadditivity seen in the present study could result from CLON and MS both acting as agonists on the same populations of receptors. However, this is unlikely since previous studies have shown that the effects of these agonists are highly selective for their respective receptors (Aghajanian, 1978; Franz et al., 1982). Therefore, these results are best interpreted as indicating that independent α_2 -adrenergic and opiate receptors converge upon a common final mechanism to increase potassium conductance. For a completely shared mechanism it would be expected that administration of a maximal concentration of one agonist would completely suppress the response to administration of the other agonist. In the present study, the summation between CLON and MS responses was examined by using drug concentrations which were 10 or more times higher than their IC_{50} s for inhibiting spontaneous activity (Andrade et al., 1983) in order to ensure the

Figure 4. Effect of near-maximal concentrations of CLON and MS and their antagonists on a manually voltage-clamped LC neuron. *Top left trace*, Action potentials elicited by intracellular current injection (+0.14 nA) in this cell. *Middle trace*, Record of the holding current (I , positive is upward). Administration of 200 nM CLON results in the appearance of an outward current. Increasing the concentration of CLON to 400 nM is only marginally effective in further increasing the amplitude of this outward current indicating that, in this cell, this concentration of CLON elicited a near-maximal effect. Administration of 2 μ M MS elicits a smaller further increase in outward current. In the presence of MS, the addition of the α_2 -antagonist PIP and the removal of CLON from the bath results in a large decrease in outward current. The blockade of both agonists by the combined administration of PIP and NALOX restores the holding current to within 0.01 nA of its initial level, attesting to the thorough removal of the effect of both agonists. (For a complete description of the experiment, refer to the text.) *Bottom trace*, Record of the membrane potential (V) during the experiment. Notice that the membrane potential at the end of the clamp is very near its initial, pre-clamp level, confirming the complete removal of the agonists which would otherwise hyperpolarize the cells even at low concentrations (Andrade and Aghajanian, 1984). Bridge balance was monitored throughout this experiment by periodic injection of 200-msec-long hyperpolarizing pulses. For purposes of clarity the resulting transients in the current record have been deleted. Similarly, the electrotonic potentials induced by these hyperpolarizing pulses (see above) were "blanked" from the record by electronically holding the voltage trace at the level just prior to the stimulus for the duration of the pulse.

TABLE I

Comparison of the individual currents (in nanoamperes) elicited by CLON and MS and by the administration of MS and CLON together in five LC neurons

	CLON	MS	Algebraic Sum (CLON + MS)	CLON and MS Co-administered	Percentage of Predicted
Mean	0.24	0.20	0.44	0.30 ^a	70.0 ^b
SD	0.09	0.12	0.20	0.12	7.3

^a $p < 0.05$ in a matched pairs t test comparing the algebraic sum of the individual currents elicited by MS and CLON with the current elicited by their co-administration.

^b $p < 0.001$ when compared in a t test with respect to the predicted value (100%).

near-maximal responses needed to permit a clear expression of nonadditivity (Deterre et al., 1982). However, in spite of the use of these high concentrations of MS and CLON, outward currents were still observed when one of these agonists was administered in addition to the other. The outward currents elicited by administration of the second agonist could have reflected the failure of the drug concentrations used to completely activate all opiate and α_2 -adrenergic receptors. Alternatively, they could have reflected the presence of a subpopulation of potassium channels activated by these receptors through independent mechanisms. Thus, although the present study shows that opiate and α_2 -adrenergic receptors share a common ionic mechanism to hyperpolarize LC neurons, the precise extent of this sharing remains to be established.

In conclusion, the results of this study suggest that opiate and α_2 -adrenergic receptors hyperpolarize LC neurons by increasing a potassium conductance which is activated by a mechanism which is at least partially shared and which could involve decreases in cAMP levels. Such a shared mechanism of action for opiates and α_2 -adrenergic receptors might not only be involved in the control of LC neuronal excitability at the somatodendritic level but might also be of relevance for the control of norepinephrine release by presynaptic receptors at LC nerve terminals (Wemer et al., 1982; Schoffeleer and Mulder, 1983).

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