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ACCELERATED COMMUNICATION

Chronic Morphine Treatment Increases Cyclic AMP-Dependent Protein Kinase Activity in the Rat Locus Coeruleus

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SUMMARY

We have studied a possible role for cyclic AMP-dependent protein kinase in mediating opiate addiction in the central nervous system by focusing on the rat locus coeruleus. This brain region is well suited for these studies because it is relatively homogeneous and because a wealth of electrophysiological and behavioral data indicate that it plays an important role in mediating the chronic effects of opiates in animals, including humans. It was found that chronic, but not acute, *in vivo* treatment of rats with morphine increased cyclic AMP-dependent protein kinase activity in the locus coeruleus with a time course that closely paralleled the time course by which locus coeruleus neurons become tolerant to and dependent on opiates, based on electrophysiological studies. Concomitant administration of the opiate receptor antagonist naltrexone was found to block the effect of chronic morphine treatment on protein kinase activity, indicating that the effect is mediated via specific activation of opiate receptors. In contrast, chronic morphine treatment did not alter protein kinase activity in several other brain regions studied, including the neostriatum, frontal cortex, and dorsal raphe. We propose that the observed increase in cyclic AMP-dependent protein kinase activity in the locus coeruleus contributes to the biochemical basis of opiate addiction.

The mechanisms by which chronic opiate treatment induces states of tolerance and dependence in target neurons remain unknown (1). One hypothesis, derived originally from experiments on cultured neuroblastoma × glioma cells, involves cyclic AMP (2–5). Acutely, opiates, through the activation of specific opiate receptors coupled to G_i (6), inhibit adenylate cyclase activity in the cells and thereby decrease cellular cyclic AMP levels (2–4). During chronic exposure of the cells to opiates, cellular adenylate cyclase activity recovers toward control levels, and subsequent removal of opiates leads to a dramatic increase in adenylate cyclase activity far above control levels (2–4). It has been proposed (2, 3) that, during chronic opiate exposure, cells increase their adenylate cyclase activity in an attempt to overcome chronic opiate inhibition of the enzyme. According to this scheme, the increase in adenylate cyclase activity seen with chronic opiate treatment represents a biochemical equivalence of "tolerance" and "dependence," and the overshoot in adenylate cyclase activity seen following cessation of treatment represents a biochemical equivalence of "withdrawal."

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The cyclic AMP hypothesis of opiate addiction remains controversial, largely because attempts to extend the studies on chronic opiate effects to the central nervous system have encountered difficulties (1, 7). One explanation for the difficulties in demonstrating consistent chronic effects of opiates on neuronal adenylate cyclase activity may be the heterogeneity of the brain regions studied; for example, the neostriatum, cerebral cortex, and cerebellum may contain too many types of neurons for opiate-induced changes in adenylate cyclase activity in any one neuronal cell type to be detectable.

Even more striking than the difficulty in demonstrating chronic effects of opiates on adenylate cyclase in brain is the almost complete lack of investigation of other steps in the cyclic AMP pathway as possible targets for chronic opiate action. Thus, it is known that most, if not all, of the effects of cyclic AMP on neuronal function are achieved through the activation of cyclic AMP-dependent protein kinase and the subsequent phosphorylation of substrate proteins for the protein kinase (8). Moreover, it has been proposed that protein phosphorylation is a final common pathway through which a large number of regulatory agents, including, perhaps, opiates, influence neuronal function (8).

We chose to study the regulation of cyclic AMP-dependent

ABBREVIATIONS: LC, locus coeruleus; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; SDS, sodium dodecyl sulfate; PKI, protein kinase inhibitor.

protein kinase by chronic morphine treatment in the rat LC. The LC is an anatomically well-defined, relatively homogeneous brain region that contains cells bodies of virtually one type of neuron—the noradrenergic neuron (9). The LC projects diffusely to most parts of the brain and is responsible for almost all of the noradrenergic innervation of the frontal cortex. The effects of many types of neurotransmitters and drugs, including opiates, on LC neurons have been well characterized electrophysiologically. Acutely, opiates depress the firing rate of LC neurons, an effect that appears to be achieved through G-proteins (10–13). A role for cyclic AMP in mediating part of this depressant action of opiates in the LC has been supported by some studies (10–12), but not by others (13). Chronically, LC neurons become tolerant to the acute depressant effects of opiates, as their firing rate recovers toward control levels upon chronic treatment; these neurons also develop dependence on opiates, as abrupt cessation of opiate treatment leads to withdrawal manifested by excessive firing of the neurons *in vivo* (14, 15). These changes in LC neuronal excitability appear to play important roles in mediating many of the acute and chronic effects of opiates in whole animals, including humans (1, 16). We report here that chronic morphine treatment increases cyclic AMP-dependent protein kinase activity in the LC, but not in several other brain regions studied. This increase in protein kinase activity, together with concomitant changes in other steps in the cyclic AMP system (see Refs. 17 and 18, and below), may mediate some of the chronic effects of opiates on LC neuronal excitability.

Materials and Methods

***In vivo* drug treatments.** Male Sprague-Dawley rats were used in these studies. Morphine pellets (National Institute on Drug Abuse, Rockville, MD), containing 75 mg of morphine base, were implanted subcutaneously in rats (initial weight 150 g) at the rate of one pellet per day for 1–8 days. Rats were used the day following the last implantation, unless otherwise specified. Such morphine treatment has been shown to produce states of tolerance and dependence in rats, based on behavioral criteria (19). The development of similar degrees of opiate addiction in rats used in this study was confirmed by monitoring the development of tolerance to acute morphine analgesia using standard procedures (19). The standard protocol for chronic morphine treatment was 5 days of treatment, with rats used on day 6, as these conditions have been shown to produce high degrees of tolerance and dependence of LC neurons to morphine, based on electrophysiological criteria (14); these conditions were kindly confirmed in our rats by Drs. K. Rasmussen and G. K. Aghajanian (data not shown). Initially, control rats underwent identical surgery but were not implanted with pellets. As this sham treatment was found to have no effect on the final results, control rats did not receive sham treatment in later experiments.

Concomitant treatment of rats with naltrexone and morphine was achieved by implanting 10-mg naltrexone pellets (Innovative Research, Toledo, OH) subcutaneously along with morphine pellets (as above) at distinct sites. Such naltrexone treatment is known to block the analgesic effects of acute morphine (20), and was shown to block the development of opiate tolerance to acute morphine analgesia (data not shown).

Protein kinase assays. Brains were removed rapidly from decapitated rats and cooled immediately in ice-cold, oxygenated buffer (126 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM D-glucose, pH 7.4). Coronal sections, 0.5 mm thick, at the level of the LC and of other brain regions were obtained by use of a McIlwain tissue slicer. LC nuclei and other brain regions were excised from coronal sections by using a 15 gauge syringe needle to "punch out" individual nuclei, similar to the technique described by Palkovits (21). For large brain regions, such as the frontal cortex and neostriatum (caudate/putamen), analysis of punches yielded results equivalent to those found by analysis of larger tissue samples. Punches of brain regions were shown to contain very similar levels of protein, as determined by standard protein assays (22); therefore, all of the data obtained in this study were normalized "per punch" rather than "per mg of protein."

Isolated brain regions were sonicated (1 mg of tissue/100 μl) in 20 mM Tris, pH 7.4, 2 mM EDTA, 1 mM dithiothreitol, 50 kallikrein units/ml aprotinin, 10 μg/ml leupeptin and subjected to ultracentrifugation (120,000 × *g*) in a Beckman Airfuge for 10 min. The resulting supernatants were used as soluble fractions of the tissue specimens. The resulting pellets were resonicated in the original volume of the same buffer and were used as particulate fractions of the tissue specimens. Aliquots of the fractions were then used for quantitation of cyclic AMP-dependent protein kinase activity by use of standard assays (23), with some modifications. Briefly, duplicate 5-μl aliquots of particulate or soluble fractions were incubated for 3 min at room temperature in a final volume of 50 μl in phosphorylation assay buffer (final concentrations: 50 mM Tris, pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 0.05% Nonidet P-40, 10 mM dithiothreitol) containing 15 μg of histone f_2b (Sigma) and 50 μM [γ-^{32}P]ATP (4–8 × 10^6 cpm/nmol, New England Nuclear) in the absence or presence of 8-bromo-cyclic AMP (5 μM) or PKI (5 μg/tube; Sigma). In some experiments, morphine sulfate (100 μM) was included in the reaction mixture. Following the incubation period, the phosphorylation mixture was blotted on 2 × 2 cm squares of P81 phosphocellulose filter paper (Whatman). The filter paper squares were washed for 1–2 hr at room temperature with tap water and air dried. The ^{32}P contained in the filter paper squares was then quantitated by liquid scintillation spectrophotometry. Under the assay conditions used, cyclic AMP-dependent protein kinase activity was linear between 1 and 5 min of incubation over a 5-fold range of tissue concentrations. The observed increase in cyclic AMP-dependent protein kinase activity in LC from morphine-treated rats was not dependent on time of incubation or tissue concentration, as similar increases were observed over the linear range of incubation times and tissue concentrations. In some experiments, the accuracy of the blotting method was confirmed by analyzing reaction mixtures by SDS-polyacrylamide gel electrophoresis and autoradiography as described in the next section. It was found that the morphine-induced increase in cyclic AMP-dependent protein kinase activity was similar by the blotting and gel methods. Cyclic AMP-stimulated protein kinase activity was completely inhibited by PKI in tissue fractions prepared from control and morphine-treated rats. In addition, no cyclic GMP-dependent protein kinase activity was observed in the LC under the assay conditions used in control or morphine-treated samples.

Previous studies on the regional distribution of cyclic AMP-dependent protein phosphorylation in rat brain showed that the pons and midbrain contained much lower levels of cyclic AMP-dependent protein kinase activity than the cerebral cortex and neostriatum (24). Initial experiments in the present study using similar assay conditions confirmed this finding: the LC, dorsal raphe, and whole coronal sections of pons and midbrain showed much lower levels of cyclic AMP-dependent protein kinase activity compared to the frontal cortex and neostriatum. Subsequent experiments demonstrated that such low levels of protein kinase activity are partly due to *in vitro* assay conditions. It was found that the inclusion of a low concentration (0.05%) of the nonionic detergent Nonidet P-40 and of a high concentration (10 mM) of the reducing agent dithiothreitol in the protein kinase assays restored levels of protein kinase activity in the LC and dorsal raphe toward levels seen in the neostriatum and frontal cortex, which were largely unaffected by the new conditions. Levels of phosphorylation in whole coronal sections of pons and midbrain were also enhanced by these conditions, although they remained significantly below those of individual brainstem nuclei. Nonidet P-40 and dithiothreitol were therefore used routinely in the present study. The mechanism by which these compounds restore protein kinase activity in brainstem nuclei is un-

known, but such compounds have been shown previously to restore maximal levels of phosphorylation of individual phosphoproteins in nervous tissue extracts (25). The specific activities of basal and cyclic AMP-dependent protein kinase in brain regions from control rats, found using the new assay conditions, are shown in Table 1.

Endogenous phosphorylation assays. Aliquots of crude homogenates or of particulate and soluble tissue fractions prepared as described above were analyzed by endogenous phosphorylation (see Ref. 24). Nonidet P-40 and dithiothreitol were included in these assays because, as discussed in the previous section, their presence was required for optimal endogenous phosphorylation in the LC and other brain stem nuclei and did not affect that in the other brain regions studied. Aliquots (containing 5–20 μg of protein) were incubated at 30° in a final volume of 100 μl in phosphorylation assay buffer (see above) containing 5–8 μM [γ-^{32}P]ATP (4–8 × 10^7 cpm/nmol) in the presence or absence of 8-bromo-cyclic AMP (5 μM) or PKI (5 μg/tube). Sixty-, 15-, and 90-sec incubation periods were used for crude homogenates, particulate fractions, and soluble fractions, respectively. Phosphorylation reactions were terminated by addition of 25 μl of (final concentrations) 50 mM Tris, pH 6.7, 4% glycerol, 2% SDS, 2% 2-mercaptoethanol, bromophenol blue as a marker. The samples were boiled for 2 min and subjected to one-dimensional SDS-polyacrylamide gel electrophoresis as described (24), using 7.5% acrylamide, 0.3% bis-acrylamide in the resolving gels. Resulting gels were dried and autoradiographed.

Results

The effect of *in vivo* morphine treatment on cyclic AMP-dependent protein kinase activity in isolated LC nuclei was determined after acute and chronic treatment. For acute treatment, animals were sacrificed 2 hr after the implantation of one morphine pellet, by which time LC neurons are known to be inhibited maximally by the absorbed morphine (14). For chronic treatment, animals were sacrificed the day after 5 days of treatment (one morphine pellet implanted per day for 5 consecutive days), conditions known to induce opiate tolerance and dependence in LC neurons (14). Fig. 1 shows the effect of such treatments on protein kinase activity, measured by a standard histone phosphorylation assay (23) with modifications that were found to be necessary for optimal analysis of protein kinase activity in brainstem nuclei (see Materials and Methods). It was found that chronic, but not acute, morphine treatment increased cyclic AMP-dependent protein kinase activity in both the particulate and soluble fractions of the LC. Regulation of protein kinase activity by chronic treatment appeared to be due to specific morphine action at opiate receptors, since concomitant treatment of rats with naltrexone, a specific and long-acting opiate receptor antagonist, blocked this effect (Fig. 1).

Increased levels of cyclic AMP-dependent protein kinase activity observed in the LC were found to be reflected in increased levels of phosphorylation of LC phosphoproteins in endogenous phosphorylation assays. Thus, chronic morphine treatment increased the overall level of cyclic AMP-stimulated phosphorylation of endogenous phosphoprotein substrates in the LC, as analyzed by one-dimensional SDS-polyacrylamide gel electrophoresis. This increase was observed in crude homogenates of the LC (Fig. 2), as well as in particulate and soluble fractions of this brain region (separate gels not shown due to space considerations). In contrast, acute morphine treatment, or the addition of morphine directly to endogenous phosphorylation assays *in vitro*, failed to produce this effect (data not shown). As can be seen in Fig. 2 (compare +protein kinase inhibitor and +cyclic AMP *lanes*), changes in the endogenous phosphorylation level of specific phosphoproteins, apart from the overall increase observed in cyclic AMP-dependent protein phosphorylation, were not evident by one-dimensional analysis.

TABLE 1

Cyclic AMP-dependent protein kinase activity in the LC and other regions of rat brain

Brain regions were dissected from control rat brains, and particulate and soluble fractions were assayed for cyclic AMP-dependent protein kinase activity as described under Materials and Methods. "Basal activity" represents protein kinase activity measured in the presence of PKI and reflects the activity of protein kinases other than cyclic AMP-dependent protein kinase under basal conditions. "Cyclic AMP-dependent activity" represents the difference between protein kinase activity measured in the presence of cyclic AMP and that measured in the presence of PKI. This difference is a measure of the total cyclic AMP-dependent protein kinase activity present in the tissue fractions. Data represent the means of determinations in four rats.

Brain region	Basal activity	Cyclic AMP-dependent activity
	nmol/min/mg protein	
LC		
Particulate	0.21	0.66
Soluble	0.29	0.85
Neostriatum		
Particulate	0.26	1.02
Soluble	0.37	2.16
Frontal cortex		
Particulate	0.22	0.65
Soluble	0.29	0.96
Dorsal raphe		
Particulate	0.26	0.78
Soluble	0.31	0.75
Pons (whole)		
Particulate	0.19	0.44
Soluble	0.20	0.43

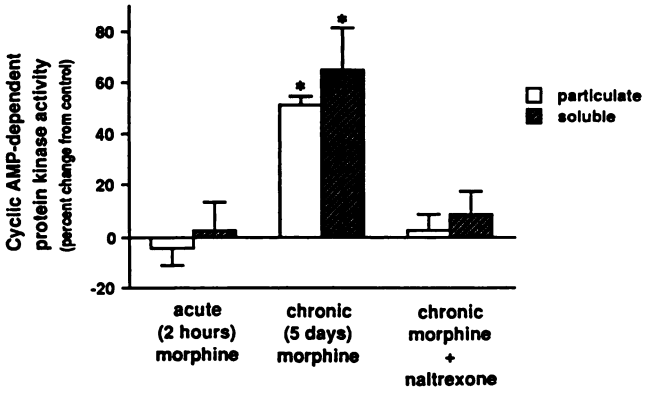

Fig. 1. Regulation of cyclic AMP-dependent protein kinase by acute and chronic morphine treatment in the rat LC. Rats received *in vivo* morphine treatment via subcutaneous pellet implantation under three conditions: 1) *acute*, one morphine pellet and the rats were used 2 hr later; 2) *chronic*, one morphine pellet per day for 5 days and the rats were used on day 6; or 3) *chronic plus naltrexone*, one morphine pellet plus one naltrexone pellet per day for 5 days and the rats were used on day 6. Control rats either underwent identical surgery but without pellet implantation or received no treatments. LC nuclei were dissected from isolated brains, and particulate and soluble fractions were assayed for cyclic AMP-dependent protein kinase activity as described under Materials and Methods. Data were calculated as the difference between protein kinase activity measured in the presence of cyclic AMP and that measured in the presence of PKI. This difference is a measure of total cyclic AMP-dependent protein kinase activity present in the tissue fractions (8). Data are expressed as per cent change from control, and error bars represent standard error. The number of animals used varied from 3 to 8. *, $p < 0.01$ by χ^2 test.

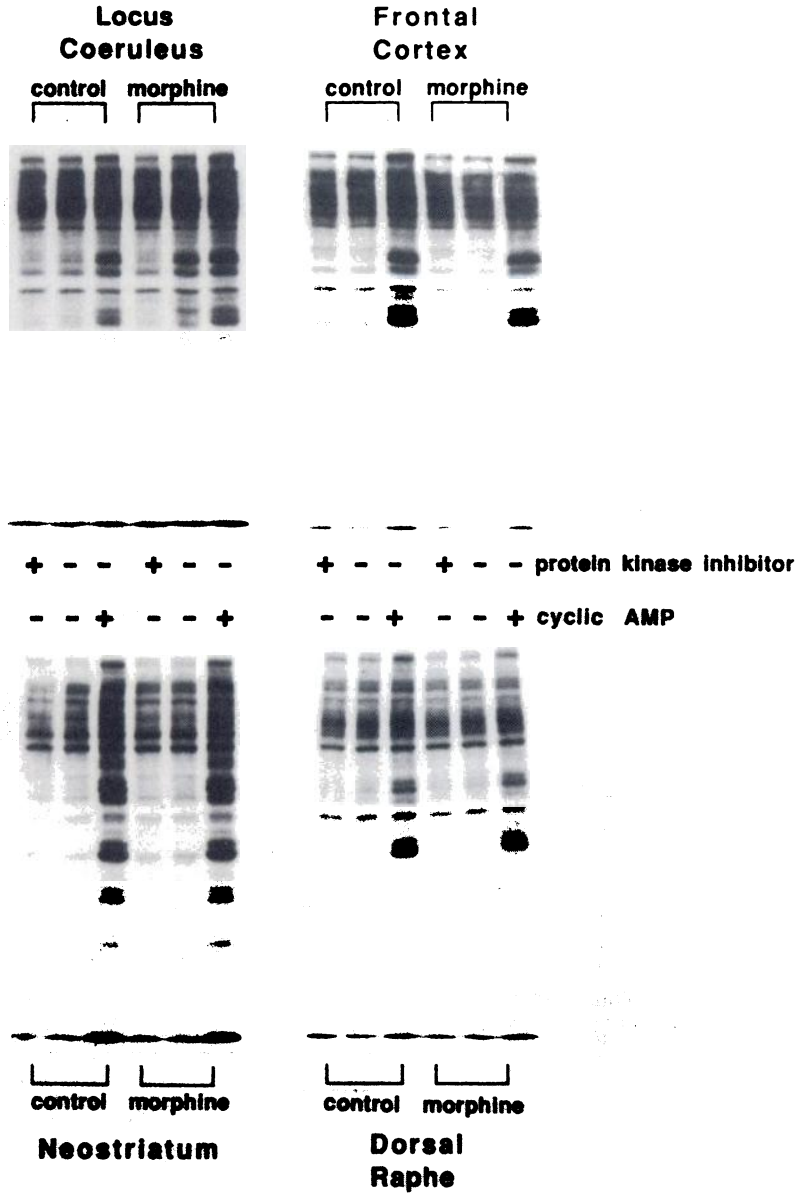

Fig. 2. Autoradiograms showing the effect of chronic morphine treatment on endogenous cyclic AMP-dependent protein phosphorylation in the LC and other regions of rat brain. Rats received chronic *in vivo* morphine treatment as described in the legend to Fig. 1. LC nuclei, neostriatum (caudate/putamen), frontal cortex, and dorsal raphe nuclei were dissected from isolated brains, and crude homogenates were analyzed by endogenous phosphorylation in the presence of PKI (5 μg/100 μl) (a specific inhibitor of cyclic AMP-dependent protein kinase), 8-bromo-cyclic AMP (5 μM), or with no further additions as indicated in the figure. The samples were then subjected to SDS-polyacrylamide gel electrophoresis and autoradiography as described under Materials and Methods. Photographs of resulting autoradiograms are shown.

A more detailed analysis of the effect of morphine on cyclic AMP-dependent protein kinase activity in the LC as a function of duration of treatment is shown in Fig. 3. Protein kinase activity was unaltered after 1 day of treatment, was increased by about 20–30% after 3 days, and showed maximal increases, of about 55–65%, after 5 or 8 days. This time course of increased cyclic AMP-dependent protein kinase activity closely parallels the time course by which rat LC neurons become tolerant to, and dependent on, morphine, as seen in electrophysiological experiments (14, 15).

The increase in cyclic AMP-dependent protein kinase activity produced by chronic morphine treatment was specific to the LC among the brain regions studied (Table 2, Fig. 2). No change in protein kinase activity was observed in either the particulate or soluble fraction of the neostriatum (caudate/putamen), frontal cortex, or dorsal raphe, the latter a serotonergic brainstem nucleus located 1–2 mm from the LC in the rat brain. Protein kinase activity was also unaltered in fractions of pons cross-sections from which LC punches were obtained, and in fractions of punches taken just lateral or just medial to the LC.

In these experiments, cyclic AMP-dependent protein kinase activity was calculated as the difference between protein kinase activity measured in the presence of cyclic AMP and that measured in the presence of protein kinase inhibitor (PKI), a specific inhibitor of cyclic AMP-dependent protein kinase. [PKI was included in the assay in order to inhibit the small amount of cyclic AMP-dependent protein kinase that is active in the absence of cyclic AMP under basal conditions (8).] The level of cyclic AMP-independent protein kinase activity, i.e., protein kinase activity detected in the presence of PKI, was not altered by acute or chronic morphine treatment in any of the brain regions studied (data not shown).

The inability of 1 day, and the ability of 5 days, of morphine treatment to increase protein kinase activity in the LC can be

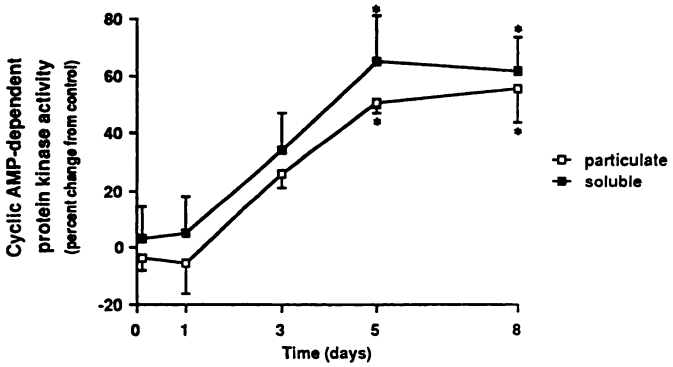

Fig. 3. Time dependence of the regulation by morphine of cyclic AMP-dependent protein kinase in the rat LC. For the earliest time point shown in the figure, rats received acute *in vivo* morphine treatment as described in the legend to Fig. 1. Other rats received *in vivo* morphine treatment via subcutaneous pellet implantations at a rate of one pellet per day for 1–8 days and were used the day following the last implantation. Control rats either underwent identical surgery but without pellet implantations or received no treatments. LC nuclei were dissected from isolated brains, and particulate and soluble fractions were assayed for cyclic AMP-dependent protein kinase activity as described under Materials and Methods. Data were calculated as the difference between protein kinase activity measured in the presence of cyclic AMP and that measured in the presence of PKI. This difference is a measure of total cyclic AMP-dependent protein kinase activity present in the tissue fractions. Data are expressed as per cent change from control. Error bars represent standard error. The number of animals used varied from 3 to 8. *, $p < 0.01$ by χ^2 test.

TABLE 2

Region-specific regulation of cyclic AMP-dependent protein kinase by chronic morphine treatment

Rats received chronic *in vivo* morphine treatment as described in the legend to Fig. 1, and particulate and soluble fractions of isolated LC nuclei and of other brain regions were assayed for cyclic AMP-dependent protein kinase activity as described under Materials and Methods. Data were calculated as the difference between protein kinase activity measured in the presence of cyclic AMP and that measured in the presence of PKI. This difference is a measure of total cyclic AMP-dependent protein kinase activity present in the tissue fractions. Data are expressed as per cent of control ± standard error, with *N* in parentheses. Actual specific activities of cyclic AMP-dependent protein kinase in brain regions from morphine-treated rats can be derived from the specific activities of those from control rats shown in Table 1.

Brain region	Cyclic AMP-dependent protein kinase activity	
	Particulate	Soluble
LC	151 ± 4 (8)*	165 ± 16 (8)*
Neostriatum	102 ± 7 (4)	108 ± 8 (4)
Frontal cortex	93 ± 6 (4)	93 ± 5 (4)
Dorsal raphe	100 ± 4 (3)	106 ± 11 (3)
Pons cross-sections minus the locus coeruleus	102 ± 1 (3)	101 ± 3 (3)
Punches lateral to the locus coeruleus	117 ± 10 (3)	108 ± 9 (8)
Punches medial to the locus coeruleus	90 ± 7 (3)	112 ± 7 (3)

*$p < 0.01$ by χ^2 test.

explained in two ways. One possibility is that chronic and persistent exposure to morphine is necessary to increase protein kinase activity. Another possibility is that 1 day of treatment is sufficient to induce this change, but that several days are needed in order for the effect to become manifested. In favor of the former interpretation is the observation that cyclic AMP-dependent protein kinase activity in the LC was at control levels in rats that had received 1 day of morphine treatment but were analyzed after a 5-day delay [100 ± 6 (3) and 98 ± 2% (3) of control ± SE (*N*) in particulate and soluble fractions, respectively]. Furthermore, the action of chronic morphine treatment on protein kinase activity was not an artifact of exogenous morphine being retained in tissue fractions, as addition of 100 μM morphine directly to kinase assays of control samples failed to alter protein kinase activity [107 ± 6 (6) and 103 ± 6% (6) of control ± SE (N) in particulate and soluble fractions, respectively].

TABLE 3

Regulation by morphine of cyclic AMP-dependent protein kinase active under basal conditions in the LC

Rats received subacute or chronic *in vivo* morphine treatment as described in the legend to Fig. 3, and particulate and soluble fractions of isolated LC nuclei were assayed for cyclic AMP-dependent protein kinase activity as described under Materials and Methods. Data were calculated as the difference between protein kinase activity measured under basal conditions (no further additions) and that measured in the presence of PKI. This difference is a measure of that portion of cyclic AMP-dependent protein kinase that is active in tissue fractions in the absence of exogenous cyclic AMP; that is, the amount of free catalytic subunit of the protein kinase present in those fractions (8). In control samples, this value showed some variability among different experiments but typically represented about 10% of the total cyclic AMP-dependent protein kinase activity present in the tissue fractions. Data are expressed as per cent of control ± standard error, with *N* in parentheses.

	Cyclic AMP-dependent protein kinase active under basal conditions	
	Particulate	Soluble
Subacute (1 day)	97 ± 3 (4)	97 ± 5 (4)
Chronic (5 days)	120 ± 4 (4)*	118 ± 7 (4)*

*$p < 0.05$ by χ^2 test.

Cyclic AMP-dependent protein kinase holoenzyme is composed of two types of subunit, regulatory subunit, designated R, and catalytic subunit, designated C (8). Cyclic AMP activates the holoenzyme by binding to R and thereby causing dissociation of the holoenzyme into free R and free C subunits. Free C subunits are catalytically active. Preliminary evidence indicates that the morphine-induced increase in protein kinase activity in the LC is associated with increased levels of free C. Thus, chronic morphine treatment produced a small, but significant, time-dependent increase in protein kinase activity measured under basal conditions (without further additions) compared to protein kinase activity measured in the presence of PKI (Table 3). This was the case in both the particulate and soluble fractions of the LC. This phenomenon is also evident in Fig. 2, where it can be seen that the level of endogenous phosphorylation in LC homogenates is unchanged in the presence of PKI, but increased under basal conditions (Fig. 2, *middle lanes*), as well as in the presence of exogenous cyclic AMP. The observed increase in free C could reflect increased levels of C without concomitant increases in R. Alternatively, it could reflect increased levels of both C and R with a concomitant increase in the fraction of the enzyme that exists in the dissociated state. The validity of these interpretations must be studied further by more direct quantitation of C and R.

Discussion

The key finding of this study is that chronic morphine treatment increases cyclic AMP-dependent protein kinase activity in the particulate and soluble fractions of the LC, but not in fractions of other brain regions studied. This increase in protein kinase activity in the LC can be viewed as a negative feedback response to chronic opiate action. Acutely, opiates are known to inhibit adenylate cyclase activity in a number of brain regions (see Refs. 1, 5, 26, and 27), including the LC (17),

and such inhibition of the enzyme would be expected to lead to decreased levels of cyclic AMP and to decreased levels of activated cyclic AMP-dependent protein kinase. The increase in protein kinase activity observed in response to chronic opiate treatment may represent an attempt by LC neurons to compensate for and overcome this chronic decrease in levels of activated protein kinase.

A striking aspect of the present study is the region-specific nature of the chronic effects of morphine. One possible explanation is that the relative homogeneity of the LC, compared to the neostriatum and frontal cortex, enables specific biochemical effects to be detected in the former. This interpretation is supported by electrophysiological observations that the latter brain regions contain neurons that respond to opiates in different ways: some neurons are inhibited, others are excited, and still others are unaffected (28, 29), whereas neurons in the LC show only one type of response, namely, inhibition (10–13). Another possible explanation for the regional specificity of the effects of morphine, not incompatible with the first, is that LC neurons are different from some other neurons in their responsiveness to opiates. This interpretation is supported by the observation that morphine did not regulate cyclic AMP-dependent protein kinase activity in the dorsal raphe, which, like the LC, is a relatively homogeneous brain region, but, unlike the LC, contains serotonergic neurons that do not appear to develop tolerance and dependence to opiates as determined by electrophysiological studies.[1] These results also indicate that chronic regulation by morphine of cyclic AMP-dependent protein kinase does not appear to be associated with a particular opiate receptor subtype. Thus, the LC, like the dorsal raphe, contains primarily μ and κ opiate receptors, whereas the neostriatum and frontal cortex contain μ, κ, and δ opiate receptors (30, 31).

Recently, chronic morphine treatment was found in related studies to also increase: (a) the level of basal, as well as GTP- and forskolin-stimulated, adenylate cyclase activity (17, 18); (b) the level of pertussis toxin-mediated ADP-ribosylation of specific G-proteins (18); and (c) the level of specific phosphoprotein substrates for cyclic AMP-dependent protein kinase;[2] and these effects, like the increase in protein kinase activity, were specific to the LC among the brain regions studied. These results, together with those of the present study, demonstrate for the first time that, during chronic exposure, morphine regulates the cyclic AMP system in LC neurons at every major step in the pathway between receptor and physiological response. We propose that the observed up-regulation of the cyclic AMP system at several levels, which would be expected to increase the excitability and firing rate of LC neurons (10–13), may be part of the biochemical basis of opiate addiction.

Acknowledgments

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[1] G. K. Aghajanian, personal communication.
[2] E. J. Nestler and J. F. Tallman, unpublished observations.

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