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Brain Research 656 (1994) 309–318

BRAIN RESEARCH

Research report

Multiple effects of long-term morphine treatment on postsynaptic β-adrenergic receptor function in hippocampus: an intracellular analysis

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Accepted 7 June 1994

Abstract

We previously reported that β-adrenergic receptors are increased in cerebral cortex and hippocampus in rats treated chronically with morphine and subsequently down-regulated after morphine withdrawal [22,23]. The changes in receptor density in hippocampus were accompanied by a corresponding super- and subsensitivity, respectively, in β-adrenergic responsiveness, as assessed electrophysiologically by measuring the ability of isoproterenol to augment population spike responses in the slice. In this study, we compared the ability of isoproterenol to reduce the Ca^{2+}-activated K^+ slow afterhyperpolarization (slow AHP) in pyramidal neurons in hippocampal slices from opiate-naive and chronic morphine-treated rats to determine whether such changes in β-adrenergic receptor function are localized postsynaptically. Chronic treatment of rats with morphine produced a 3.5-fold parallel shift to the left in the concentration–response curve for isoproterenol and reduced the EC_{50} from 4.8 ± 1.3 to 1.4 ± 0.5 nM. In contrast, sensitivity and maximal responsiveness to isoproterenol was markedly decreased in pyramidal neurons recorded in slices from morphine withdrawn animals. The concentration–response curves for inhibition of the slow AHP by carbachol or forskolin were not affected by chronic morphine treatment. However, blockade of the slow AHP by forskolin was significantly reduced in pyramidal neurons studied after morphine withdrawal. These data suggest that the increase in electrophysiological responsiveness to β-adrenergic receptor stimulation found in hippocampus after chronic morphine treatment most likely resulted from an up-regulation in postsynaptic membrane receptors, whereas alterations occurring beyond the receptor level may be involved in the desensitization that is associated with morphine withdrawal.

Key words: Chronic morphine; Morphine withdrawal; β-Adrenoceptor; Ca^{2+}-activated potassium afterhyperpolarization; Pyramidal neuron; Hippocampal slice

1. Introduction

Much evidence suggests that the central noradrenergic system arising from neurons of the pontine nucleus locus coeruleus (LC) may play an important role in opiate dependence and withdrawal (for review, see [31]). For example, naloxone-precipitated withdrawal of opiate-dependent rats dramatically increases the spontaneous firing rate of LC neurons [2,29,37], and the time course of increased LC activity closely matches that of several withdrawal behaviors [29]. In addition, administration of the α_2-adrenergic agonist clonidine suppresses the withdrawal-induced hyperactivity in LC neurons [2] and blocks several components of the morphine-withdrawal syndrome in opiate-dependent rats, monkeys and man [13,30].

Other data indicate that compensatory adaptations in postsynaptic receptors on LC target neurons occur after long-term opiate administration, and these may contribute as well to the elevation in central noradrenergic activity that underlies withdrawal phenomena. Work from this and other laboratories has shown that chronic treatment of rats with morphine increases the number of β-adrenergic receptors in cerebral cortex and hippocampus, without changing the affinity of these receptors for agonists or antagonist ligands [16,22,23]. The up-regulation in β-adrenergic receptors found after chronic morphine treatment appeared to be of functional significance, since it was accompanied by an

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SSDI 0006-8993(94)00681-2

increased electrophysiological responsiveness to β-adrenergic agonists as evidenced by an enhanced ability of isoproterenol to augment CA1 population spike responses evoked by Schaffer collateral stimulation in rat hippocampal slices [22]. Conversely, withdrawal of morphine-dependent rats was associated with a decrease in the number of hippocampal β-adrenergic receptors and a corresponding reduction in electrophysiological sensitivity and maximal responsiveness to isoproterenol [22]. The latter changes could represent a compensatory down-regulation in the β-adrenergic receptor system in response to the exaggerated synaptic release of norepinephrine that occurs during withdrawal [7,9,29]. Moreover, the emergence of such subsensitivity in postsynaptic β-adrenergic receptors, if shown to be of a persistent nature, may provide the cellular basis for the hypofunction in central noradrenergic and peripheral sympathetic systems that characterizes the secondary or protracted abstinence syndrome [21].

The population spike response to Schaffer collateral stimulation that is recorded extracellularly from the CA1 cell layer represents the summated firing of large numbers of pyramidal neurons [4]. Measurements that are derived from monitoring population spike amplitude are sensitive to both presynaptic and postsynaptic influences that affect the probability of pyramidal neuron discharge to this excitatory input. The increases in population spike amplitude produced by the application of β-adrenergic agonists are known to occur independent of changes in the level of synaptic activation (as reflected by the constancy of the dendritic field EPSP) [22,24]. On the other hand, activation of postsynaptic β-adrenergic receptors can directly increase the responsiveness of pyramidal neurons to depolarizing stimuli by producing blockade of the Ca^{2+}-activated K^+ current, I_{AHP} [14,18]. This conductance underlies the slow AHP that follows a burst of action potentials in pyramidal neurons and by this latter action serves normally to limit stimulation-induced repetitive firing [14,18,32]. In addition, application of β-adrenergic agents or norepinephrine to the slice has been shown to modify local inhibitory GABAergic input to pyramidal neurons and thereby could act indirectly to influence their excitability and probability of spike discharge [5,10]. Consequently, it remains unclear to what extent the alterations in ability of β-adrenergic agonists to augment population spike responses that accompany morphine dependence and withdrawal might reflect changes in 'presynaptic' receptor populations, rather than those localized on pyramidal neurons.

In the present study, intracellular recordings were obtained from pyramidal neurons in vitro in rat hippocampal slices to determine whether the changes in hippocampal sensitivity and responsiveness to β-adrenergic stimulation found after chronic morphine treatment primarily reflect alterations localized to postsynaptic receptors on pyramidal neurons. Changes in postsynaptic β-adrenergic receptor function were assessed by comparing the ability of isoproterenol to inhibit the slow AHP that follows a current-evoked burst of action potentials in neurons from opiate-naive controls and morphine-dependent rats before and after withdrawal. Similar comparisons were made of the abilities of forskolin and carbachol to reduce the slow AHP to ascertain whether any observed changes in β-adrenergic sensitivity or responsiveness after chronic opiate treatment or withdrawal might involve alterations occurring beyond the receptor level. β-Adrenergic blockade of the slow AHP occurs via the G_s-mediated stimulation of adenylate cyclase and the resultant increase in intracellular levels of cAMP [19,32]. On the other hand, the stimulation of muscarinic receptors by carbachol leads to an increased turnover in membrane phosphoinositides and blockade of I_{AHP} (and concomitant slow AHP responses) in hippocampal pyramidal neurons via a protein kinase C-dependent signaling cascade [1,20]. Comparing the effects of chronic morphine treatment on the responses to isoproterenol and carbachol thus provided us with a means to (1) assess the specificity of long-term opiate interactions with the β-adrenergic receptor coupled adenylate cyclase system and (2) determine whether chronic opiate treatment might alter slow AHP responses by directly affecting the underlying conductance I_{AHP}. Some of these results have been presented previously in preliminary form [1].

2. Materials and Methods

Male, Sprague-Dawley rats (Charles River) weighing 250–350 g were used in this study. Rats were treated chronically with morphine pellets (containing 75 mg morphine base), implanted subcutaneously on the back flank under halothane anesthesia (4% in air). The treatment schedule was as follows: rats were implanted with one pellet on day one, three pellets on day 3, five pellets on day 5, and seven pellets on day 7. This treatment schedule has been shown previously to produce a high level of tolerance and dependence on morphine [6]. The accelerated morphine regimen was well-tolerated by all of the treated animals with rats showing no obvious behavioral impairments and maintaining body weight throughout the treatment period.

Brain slices were prepared either two or four days after the last implantation of morphine pellets, corresponding to days 9 and 11 of the schedule, respectively. In rats rendered dependent by pellet implantation, 1 mg/kg of naloxone, injected s.c., precipitated highly characteristic 'dominant' withdrawal signs when administered within 60 h after the final implants. These included hyperactivity, uncontrollable jumping and increased escape attempts, abnormal swallowing movements, diarrhea, teeth chattering and screeching on touch. Neurons that were studied in slices prepared on day 9 thus comprised the morphine-dependent group. Those tested 4 days after the last pellet implantation were designated opiate-withdrawn, based on the occurrence of a spontaneous withdrawal syndrome in the treated animals on day 10 of the schedule. The extent and duration of the

withdrawal period were determined by monitoring weight loss in the animals after cessation of opiate treatment, considered a reliable index of morphine withdrawal in rats [40]. Reductions in weight were first noted on day 10 of the schedule and peaked by day 11, with the average loss exceeding 13% (10 to 18% range, $n = 15$) of mean body weight measured over the course of morphine treatment. The withdrawn animals exhibited other signs of an abstinence syndrome such as abnormal posturing, lacrimation, seminal emissions and screeching on touch; however, the 'dominant' signs of precipitated abstinence were typically absent when administration of naloxone was delayed until after the onset of weight loss occurred.

On the test day, animals were rendered unconscious with CO_2 and sacrificed by decapitation. The brain was removed and immediately placed in ice-cold artificial cerebrospinal fluid (ACSF), containing, in mM: NaCl 124, KCl 3.5, $MgSO_4$ 1.5, NaH_2PO_4 1.0, $NaHCO_3$ 26.2, glucose 11.0, $CaCl_2$ 2.5, pregassed with 95% O_2 and 5% CO_2. The hippocampus was dissected free, and transverse slices were cut at 500 microns on a McIlwain chopper with the regio inferior and dentate gyrus placed upward. One slice was transferred immediately to the recording chamber and held submerged between two layers of nylon mesh under continuously flowing ACSF (perfused at 1 ml/min) pre-warmed to 32.5°C. The remaining slices were placed in an incubation chamber and maintained in oxygenated ACSF at room temperature. Slices were allowed to equilibrate for at least 1 h before intracellular recordings were attempted. During recordings, drugs were applied to the slice by switching the bath superfusate from normal ACSF to medium containing known concentrations of drugs.

Conventional intracellular voltage recordings were obtained from CA1 pyramidal neurons using glass microelectrodes filled with 2 M KCl (pH = 7.0) and having resistances of 70–120 MΩ. Neurons were stimulated to fire action potentials by passage of a short depolarizing current pulse through the recording electrode using a standard bridgebalanced amplifier. Recordings were made in the presence of the sodium channel blocker tetrodotoxin (TTX, 1 μM) and the voltage-dependent potassium channel blocker tetraethylammonium (TEA, 5 mM) to enhance the slow AHP responses that followed a current-evoked burst of spikes. TTX was also used to block axonal conduction, thereby eliminating possible confounding effects arising from drug actions at sites distal to the pyramidal neuron being recorded. Recordings of membrane voltage were amplified and displayed on an oscilloscope and chart recorder. The signals were also led to a computer interface which digitized the analog waveforms for analysis by a microcomputer based program (pClamp, Axon Instruments).

To quantify the inhibitory action of drugs on the slow AHP, samples of four digitized waveforms taken prior to and during drug application were averaged. The area of the slow AHP was calculated by integrating with respect to time the hyperpolarizing potential falling below baseline. The lower and upper time limits utilized for the integration of the slow AHP were the time following the current pulse when the slow hyperpolarizing potential first emerged from the decay phase of the preceding medium AHP and the time when the potential returned to the pre-pulse level, respectively. The area of the slow AHP during drug administration was expressed as a percentage of the response measured in the absence of drug for each neuron.

Data statistical analysis. The results obtained from morphine-treated rats were compared to those from opiate-naive controls using a two-way analysis of variance (ANOVA) with repeated measures on one variable (concentration). Post-hoc analysis was done using the Dunnett test to compare the treatment group means to the control group means at each drug concentration. The concentration of drug required to inhibit the control AHP by 50% (EC_{50}) was calculated using linear regression analysis for cells in which a complete concentration–response curve was obtained. The percent reduction in the response produced at each concentration of drug was expressed by normalizing each value to the maximal inhibition obtained in a given neuron and multiplying by 100. All data are presented in the text as mean ± S.E.M..

Drugs. Carbachol, (−)-isoproterenol, tetraethylammonium chloride and tetrodotoxin were purchased from Sigma Chemical Co. and forskolin was purchased from Calbiochem. Morphine pellets were obtained from the National Institute on Drug Abuse.

3. Results

3.1. Effects of chronic morphine treatment on resting and active membrane properties

The resting membrane properties of pyramidal neurons recorded in slices from chronic morphine-treated ($n = 23$, 12 animals) and morphine-withdrawn rats ($n = 17$, 12 animals) were not significantly different from those of control neurons ($n = 25$, 16 animals) (Table 1). In most control and opiate-treated neurons, passage of a 150 ms depolarizing pulse (0.5–2.0 nA, intensity) through the recording electrode evoked a series of one to three Ca^{2+} action potentials followed by a medium and subsequent slow AHP. The slow AHP was not sensitive to the presence of TEA (5 mM) in the bath, but was eliminated during perfusion of the Ca^{2+} channel blocker Cd^{2+} (200 μM) (6/6 neurons). Intracellular administration of the K^+ channel blocker Cs^+ also completely abolished the slow AHP (3/3 neurons recorded with microelectrodes containing 3M cesium acetate). The late slow hyperpolarizing potentials recorded in opiate-naive neurons were typically 7–11 mV in amplitude, 3–6 s in duration and were accompanied by a marked decrease in membrane input resistance. The properties of the slow AHP recorded in morphine-treated neurons were essentially indistinguishable from those of responses elicited in controls (Table 1). The mean durations of the slow AHP determined for all neurons within the control, chronic morphine-treated and withdrawn groups were 4098 ± 297, 3612 ± 295 and 3712 ± 196 ms, respectively. In addition, determination of the time constants of decay from a representative sampling of neurons yielded values of 1.9 ± 0.3 ms for controls ($n = 9$), 1.8 ± 0.5 ms for the chronic treated group ($n = 6$) and 2.0 ± 0.4 ms for the withdrawn group ($n = 4$).

Table 1
Comparison of resting membrane properties and Ca^{2+}-activated potassium slow AHPs in CA1 pyramidal neurons from control and chronic morphine-treated rats

	RMP (mV)	AHP Amplit. (mV)	AHP Duration (s)	AHP Decay τ (s)
Controls (25)	−70.1 ± 1.4	−6.1 ± 0.3	4.1 ± 0.3	1.9 ± 0.3
Treated (23)	−66.8 ± 1.2	−5.3 ± 0.4	3.6 ± 0.3	1.8 ± 0.2
Withdrawn (17)	−67.8 ± 2.1	−5.2 ± 0.4	3.7 ± 0.2	2.0 ± 0.3

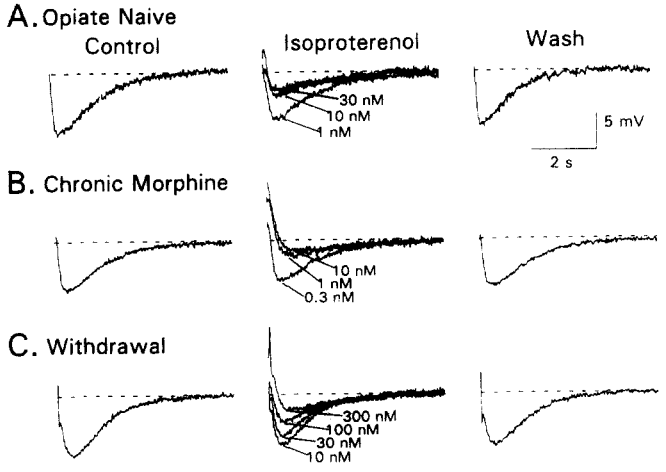

Fig. 1. A–C: representative records from three experiments comparing sensitivity to isoproterenol-induced blockade of the Ca^{2+}-activated K^+ slow AHP in pyramidal neurons from an opiate-naive (A), chronic morphine-treated (B) and morphine-withdrawn rat (C). Sensitivity to the inhibitory effect of isoproterenol was increased after chronic morphine treatment, but it was decreased and the maximal effect of the agonist reduced in the withdrawn neuron, compared to the opiate naive-control. Each trace is the computerized average of slow AHP responses elicited by 4 stimulation trials. Resting membrane potentials were −70 mV, −69 mV and −68 mV for the neurons depicted in A, B and C, respectively.

In these experiments, slices that were prepared from chronic morphine-treated rats were maintained continuously in ACSF that did not contain morphine. No evidence of a withdrawal response (e.g. membrane depolarization or increased postsynaptic potential activity) was observed in any of the dependent neurons during intracellular recording over an 8 h period from the time of slice preparation. In previous studies, perfusion of opiate-free medium for up to 8 h or challenge with naloxone also failed to elicit any electrophysiological signs of withdrawal in hippocampal or LC neurons in slice preparations from chronic morphine-treated rats [6,42].

3.2. β-Adrenergic sensitivity and responsiveness after chronic morphine treatment

Bath application of isoproterenol reversibly reduced the slow AHP that followed a current-evoked burst of action potentials in all CA1 pyramidal neurons examined. The left voltage trace in Fig 1A. illustrates a typical slow AHP response of a control neuron elicited by passage of a 150 ms depolarizing current pulse (1.0 nA) through the recording electrode. This neuron fired a single broad Ca^{2+} action potential (not shown in this truncated record) followed by a large hyperpolarization of 11.5 mV amplitude and 4.3 s duration. Addition of 10 or 30 nM isoproterenol to the bath resulted in a large reduction in the area of the slow AHP (middle panel) which gradually reversed upon washout of the drug (right panel). The inhibition of the slow AHP by isoproterenol was concentration-dependent and typically occurred independent of changes in the resting membrane potential. Depolarizations of a few mV accompanied blockade of these afterhyperpolarizations in only 6 neurons (3 control and 3 dependent). However, in roughly 25% of control (6/25) and chronic morphine-treated neurons (7/23) administration of the β-adrenergic agonist had a biphasic effect on these responses, with an increase in slow AHP amplitude and duration occurring upon repeated testing of the

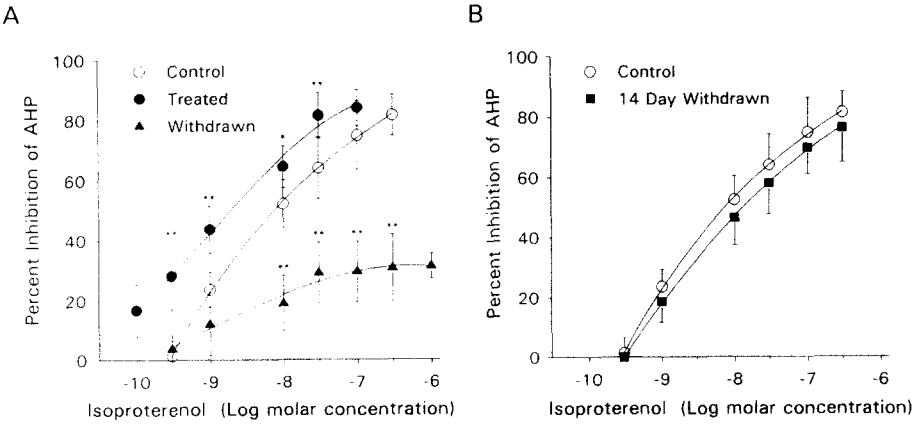

Fig. 2. A: concentration–response curves for inhibition of the slow AHP by isoproterenol in pyramidal neurons from control (open circles), chronic morphine-treated (filled circles) and morphine-withdrawn rats (filled triangles). Chronic morphine treatment shifted the concentration–response curve 3.5-fold to the left and reduced the EC_{50} for isoproterenol from 4.8 ± 1.3 nM to 1.4 ± 0.5 nM. In contrast, the EC_{50} for isoproterenol was increased to 71.1 ± 29.6 nM and the maximal effect of the agonist was reduced in neurons studied after morphine withdrawal. B: comparison of isoproterenol concentration–response curves from opiate-naive controls (open circles) and morphine-withdrawn neurons (filled squares) studied 7 days following the last pellet implantation. Symbols represent the mean values and error bars the s.e.m of determinations in 6–8 neurons. * $P < 0.05$, ** $P < 0.01$, compared to control.

Table 2
Comparison of EC_{50} values for inhibition of the slow AHP by isoproterenol, forskolin and carbachol before and after chronic morphine treatment

	Isoproterenol (nM)	Forskolin (μM)	Carbachol (μM)
Controls	4.5 ± 1.2 (5)	1.7 ± 0.4 (10)	1.3 ± 0.3 (7)
Treated	1.4 ± 0.5 (6)	1.2 ± 0.2 (5)	1.9 ± 0.6 (7)
Withdrawn	71.1 ± 29.6 (7) *	6.6 ± 2.1 (6) *	2.1 ± 0.6 (4)

Values represent the means ± S.E.M. for the number of experiments shown in parentheses.
* Significantly different from control at $P < 0.05$ by Mann–Whitney U-test.

drug at higher concentrations (above 100 nM, see below).

Sensitivity of pyramidal neurons to the inhibitory effect of isoproterenol on the slow AHP was increased after chronic morphine treatment (Table 2). It can be seen from the voltage traces depicted in Fig. 1 that 1 nM isoproterenol had a more potent inhibitory effect in the neuron from the chronic morphine-treated animal (Fig. 1B), producing 80% inhibition of the slow AHP, compared to 26% inhibition of the response in the control neuron (Fig. 1A). In addition, the maximal effect was obtained at a lower concentration of isoproterenol in the treated neuron (97% inhibition of the AHP at 10 nM), whereas 3- to 10-fold higher concentrations of the agonist were required to achieve comparable effects (86% and 89% inhibition of the response, respectively) in the opiate-naive cell. Fig. 2A compares the concentration–response curves for inhibition of the slow AHP by isoproterenol in control ($n = 8$) morphine-treated ($n = 8$) and morphine-withdrawn groups of neurons ($n = 6$). Chronic treatment of rats with morphine resulted in a 3.5-fold parallel shift to the left in the concentration–response curve for isoproterenol (Fig. 2A) with a significant group effect ($F_{1,22} = 18.106$, $P < 0.0003$, repeated measures ANOVA). The mean inhibitory effect produced was significantly greater in the morphine-dependent neurons compared to controls at all concentrations of the agonist, except for 100 nM which yielded maximal effects in both groups. However, the EC_{50} for inhibition by isoproterenol was reduced only slightly after chronic morphine treatment from 4.8 ± 1.3 nM to 1.4 ± 0.5 nM ($P < 0.10$).

It was not always possible to examine the complete concentration–response relationship for isoproterenol in each neuron because of the occurrence of biphasic effects. Administration of isoproterenol in a 3-fold incremental series of concentrations from 0.3 to 30 nM typically resulted in graded reductions in slow AHP amplitude and area that varied directly with agonist concentration. However, when measure-ments were made in the presence of higher concentrations of agonist, the number of Ca^{2+} action potentials evoked by a depolarizing current pulse of constant amplitude and duration increased (compared to the pre-drug condition) in some control ($n = 6$) and morphine-dependent neurons ($n = 7$), and there was a corresponding enhancement in the amplitude and duration of the slow AHP. For this reason, it was not possible to obtain a reliable measure of the agonist-induced inhibition of the slow AHP with 300 nM isoproterenol in neurons from the morphine-dependent group. It seemed likely that such biphasic effects were related to a desensitization of β-adrenergic receptors, since facilitation of Ca^{2+} spike generation and slow AHP responses was not encountered when 300 nM isoproterenol was initially tested in neurons without prior exposure to lower concentrations of the agonist.

In contrast to the results obtained after chronic morphine treatment, neurons ($n = 6$) studied in slices from morphine-withdrawn rats showed a significant decrease in postsynaptic sensitivity and responsiveness to β-adrenergic stimulation (Table 2, Figs. 1C and 2A). The concentration–response curve for slow AHP inhibition by isoproterenol was shifted 20-fold to the right (measured at EC_{50} level) and downward in withdrawn neurons, compared to opiate naive controls ($F_{1,22} = 33.29$, $P < 0.0001$) (Fig. 2A). The EC_{50} for isoproterenol was increased significantly in morphine-withdrawn neurons (71.1 ± 29.6 nM, $P < 0.01$, compared to 4.8 ± 1.3 nM for controls), and the maximal responsiveness at 300 nM isoproterenol was reduced by 60% ($P < 0.01$). As shown in Fig. 1, for example, 10 nM isoproterenol inhibited the slow AHP by 75% in an opiate-naive neuron, whereas 30 and 100 nM isoproterenol produced only a 30 and 56% inhibition of the response, respectively, in a neuron recorded in a slice from a morphine-withdrawn rat. Moreover, in the latter neuron 300 nM isoproterenol failed to produce complete blockade of the slow AHP.

To determine the extent and time course of recovery from the β-adrenergic subsensitivity found in neurons from morphine-withdrawn rats, we examined the ability of isoproterenol to reduce the slow AHP in neurons ($n = 8$) 1 week after the last implantation of morphine pellets. At this time (day 14 of the schedule), 4 days had elapsed since the expression of withdrawal signs in these animals. Fig. 2B compares the concentration–response curves for inhibition of the slow AHP by isoproterenol in control neurons and neurons from chronic morphine-treated rats studied on day 14, after recovery from withdrawal. In the latter neurons, the EC_{50} for isoproterenol (EC_{50} 6.4 ± 2.2 nM) and maximal inhibitory effect produced by the β-adrenergic agonist (82.5 ± 6.8% at 300 nM) were not significantly different from values in opiate naive controls (EC_{50} 4.8 ± 1.3 nM and 81.5 ± 7.2%, $P > 0.5$). Nor were any significant group differences found at any agonist concentration tested, compared to controls.

3.3. Assessments of changes occurring beyond the receptor level

We next sought to determine whether the alterations in postsynaptic responsiveness to isoproterenol found here could be attributed simply to the up- and down-regulation in hippocampal β-adrenergic receptor density, respectively, that occurs after chronic morphine treatment and withdrawal [22]. To test this possibility, we compared the ability of forskolin to reduce the slow AHP in neurons from opiate-naive controls and chronic morphine-treated rats before and after withdrawal. Forskolin is known to directly activate the catalytic subunit of adenylate cyclase and thereby acts beyond the level of the β-adrenergic receptor to reduce the slow AHP in hippocampal pyramidal neurons [19,32]. Thus, we reasoned that if changes in the number of postsynaptic β-adrenergic receptors were solely responsible for the alterations in response to isoproterenol, then the inhibitory effect of forskolin was likely to be unchanged in neurons taken from chronic morphine-treated rats. Bath application of forskolin (0.3–30 μM) produced a concentration-dependent reduction in the area of the slow AHP, which reversed within 10–25 min after washout of the drug. This effect appeared to be due to a stimulation of cyclase activity, since administration of higher concentrations of 1,9-dideoxyforskolin (50–100 μM), which has non-specific effects like the parent compound but does not activate adenylate cyclase, was without effect (4/4 neurons, data not shown). Chronic treatment of rats with morphine did not alter the ability of forskolin to reduce the slow AHP (Figs. 3 and 4). The EC_{50} for forskolin was 1.2 ± 0.2 μM in neurons from morphine-dependent animals ($n = 7$), which was not significantly different from that for controls (1.7 ± 0.4 μM, $n = 6$). Nor were any significant differences detected between the mean inhibitory effects produced in the morphine-dependent neurons compared to controls over the 100-fold range of concentrations (0.3 to 30 μM) of forskolin that were tested. In contrast, in neurons studied after morphine withdrawal ($n = 6$), the ability of forskolin to reduce the slow AHP was significantly reduced (Table 2, Figs. 3 and 4). The concentration–response curve for forskolin was shifted 4-fold to the right in withdrawn neurons, compared to control (Fig. 4, $F_{1,20} = 10.998$, $P < 0.003$). Although the EC_{50} for forskolin was increased in withdrawn neurons (6.6 ± 2.1 μM vs. 1.7 ± 0.4 μM for controls, $P < 0.01$), the maximal inhibitory effect measured at 30 μM forskolin was similar between the two groups.

In a final series of experiments, we compared the inhibitory effects of muscarinic receptor activation on the slow AHP in control and chronic morphine-treated neurons. The aim of these experiments was to determine whether the alterations in β-adrenergic receptor function induced by chronic morphine treatment were confined to the β-adrenergic receptor-coupled adenylate cyclase system or might be generalized to other intracellular signaling cascades that are negatively cou-

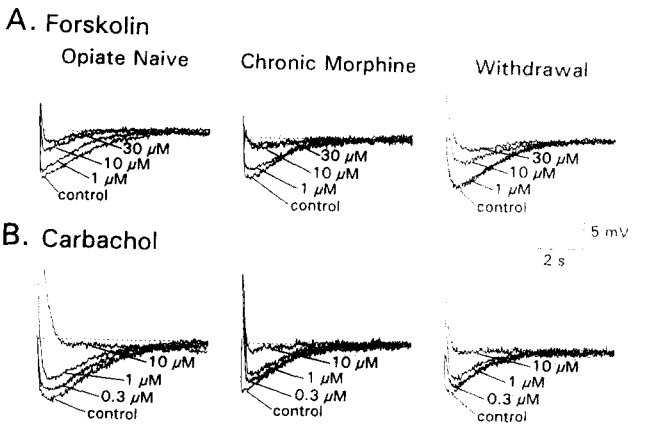

Fig. 3. A: representative records from 3 experiments comparing the ability of forskolin in varying concentrations to inhibit the slow AHP in neurons from an opiate-naive (traces at left), chronic morphine-treated (middle traces) and morphine-withdrawn rat (traces at right). Sensitivity to forskolin was similar in the chronic morphine-treated and control neuron, but was reduced in the withdrawn neuron (comparisons made at 1 and 10 μM) without change in maximal effect (measured at 30 μM). B: records from a similar series of 3 experiments in which blockade of the slow AHP by varying concentrations of carbachol was compared between control (traces at left) and chronic morphine-treated neurons studied before (middle traces) and after withdrawal (traces at right). Quantitative analyses of the effects produced over a 30-fold range of agonist concentrations (0.3 to 10 μM) showed that inhibition of the slow AHP by muscarinic receptor activation was unaffected after chronic morphine treatment or withdrawal. Traces shown are computerized averages of 4 stimulus trials. Resting membrane potentials ranged from −68 mV to −71 mV for the neurons depicted.

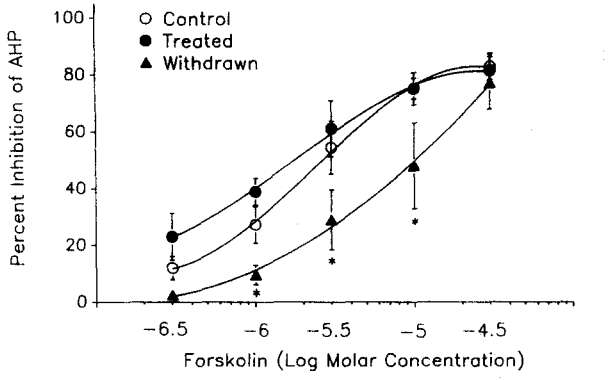

Fig. 4. Concentration–response curves for forskolin-induced inhibition of the slow AHP in neurons from control (open circles), chronic morphine-treated (filled circles) and withdrawn rats (filled triangles). Sensitivity to forskolin was unchanged after chronic morphine treatment, but the concentration–response curve was shifted 4-fold to the right and the EC_{50} increased from 1.7 ± 0.4 μM to 6.6 ± 2.1 μM ($P < 0.05$) in neurons from morphine withdrawn rats. Symbols represent the mean values and error bars the S.E.M. of determinations in 6–7 neurons. * $P < 0.05$.

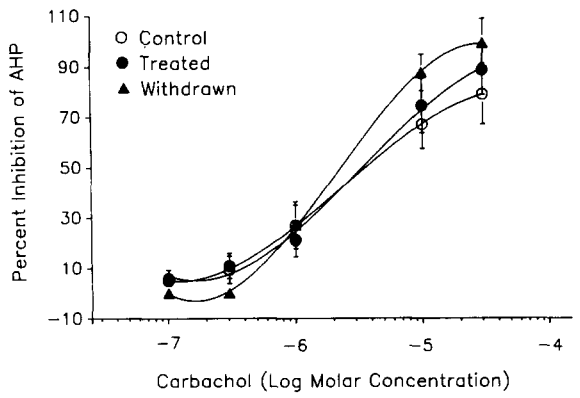

Fig. 5. Comparison of concentration–response curves for carbachol-induced inhibition of the slow AHP in neurons from opiate-naive (open circles), chronic morphine-treated (filled circles) and morphine-withdrawn rats (filled triangles). Blockade of the AHP that resulted from the activation of muscarinic receptors was unaffected by chronic treatment of rats with morphine or morphine withdrawal. Symbols represent mean values and error bars the S.E.M. of determination in 58 neurons.

pled to I_{AHP}. We first sought to establish that muscarinic and β-adrenergic receptors modulate the same slow Ca^{2+}-activated K^+ conductance by determining whether the inhibitory effects of carbachol and isoproterenol on the slow AHP were non-additive. In all 6 neurons examined (3 controls and 3 chronic morphine-treated), there was occlusion of the inhibitory response produced by carbachol (3 μM) or isoproterenol (30 nM) when one of the drugs was applied in the presence of a near saturating concentration of the other (data not shown). This result is consistent with other data in the literature which indicate that muscarinic and β-adrenergic receptors are negatively coupled to I_{AHP} in rat hippocampal pyramidal neurons [11,14,18,20].

Neither chronic morphine treatment nor withdrawal altered the ability of carbachol to reduce the slow AHP in CA1 pyramidal neurons (Figs. 3 and 5). The EC_{50}s for AHP inhibition by carbachol in neurons from chronic morphine-treated ($n = 8$) or withdrawn rats ($n = 5$) were similar to the value obtained in opiate-naive controls ($n = 8$) (Table 2). The voltage traces in Fig. 3B directly compare the reductions in the slow AHP produced by 0.3, 1 and 10 μM carbachol in control (left panel), chronic morphine-treated (middle panel) and morphine-withdrawn neurons (right panel).

4. Discussion

The present results demonstrate that alterations occur in postsynaptic β-adrenergic receptor function in hippocampal pyramidal neurons after chronic morphine administration and withdrawal. The emergence of postsynaptic β-adrenergic receptor supersensitivity after chronic morphine treatment was evidenced by a 3.5-fold leftward shift in the concentration–response curve for inhibition of the Ca^{2+} activated K^+ slow AHP by isoproterenol. In contrast, neurons in slices prepared from morphine-withdrawn rats showed a marked subsensitivity to isoproterenol and a decrease in maximal response to the agonist. The latter changes in neuronal responsiveness to β-adrenergic receptor activation showed full recovery back to control levels within one week following the expression of withdrawal signs.

It is well-established that the primary response measurement employed here, i.e., blockade of the slow AHP by isoproterenol, results from activation of β-adrenergic receptors on pyramidal neurons and an elevation in intracellular levels of cAMP [14,18,19,32]. Moreover, all recordings were made in the presence of TTX (1 μM), ensuring postsynaptic isolation of the cell under study. Thus, it is reasonable to conclude that the functional alterations in β-adrenergic receptor response observed were localized postsynaptically to pyramidal neurons. The data further suggest that the up- and downregulation in β-adrenergic receptor density described previously in rat hippocampus after chronic morphine treatment and withdrawal, respectively [22], involves populations of receptors on pyramidal neurons.

The increase in neuronal sensitivity to isoproterenol found after chronic morphine treatment is in keeping with the results of earlier biochemical studies which have demonstrated an enhanced responsiveness of adenylate cyclase to β-adrenergic stimulation in several brain areas in morphine-dependent rats. Thus, chronic administration of morphine in vivo has been reported to increase cAMP accumulation by isoprenaline and norepinephrine in rat cortical slices [16] and to enhance isoproterenol-stimulated adenylate cyclase activity in monkey cerebella [25]. Prolonged exposure to morphine also increased isoprenaline-stimulated cAMP production in cultures of rat striatal neurons [38]. The latter findings are of particular interest since these cultured neurons, unlike other cellular systems routinely employed in opiate studies, predominantly contain μ-opioid receptors [39], the target of morphine action. Our findings that neuronal sensitivity and responsiveness to isoproterenol were reduced in slices from withdrawn rats are consistent with results of Nathanson and Redmond [25] who found that withdrawal of morphine-dependent monkeys is associated with decreased isoproterenol-stimulated adenylate cyclase activity. In contrast, Kuriyama found a significant increase in cortical cAMP formation by norepinephrine after naloxone-precipitated withdrawal of morphine-dependent rats [15]. The explanation for the discrepancy in results between studies is unclear, but may reflect fundamental differences in β-adrenergic receptor dynamics (and brain NE turnover) that occur in response to acute withdrawal precipitated by naloxone vs. that associated with drug abstinence.

The magnitude of the increase in postsynaptic sensitivity to isoproterenol found after chronic morphine treatment is close to the effect predicted (see [12]) for the 20% elevation in hippocampal β-adrenergic receptor density measured in our earlier binding studies [22]. The fact that the concentration–response curves for inhibition of the slow AHP by forskolin or carbachol were unaffected by chronic morphine treatment adds credence to the likelihood that changes in the number of membrane receptors primarily accounted for the increase in neuronal sensitivity to β-adrenergic stimulation in slices from morphine-dependent rats. In previous competition binding experiments, we failed to detect any significant changes either in the high affinity binding of isoproterenol or its inhibitory potency for displacement of [^{3}H]dihydroalprenolol in hippocampal membranes prepared from chronic morphine-treated or morphine-withdrawn rats, compared to naive controls [22]. Further, it should be mentioned that the major components of the adenylate cyclase cascade that mediate β-adrenergic signal transduction, including the stimulatory G protein G_s and the activities of adenylate cyclase and cAMP-dependent protein kinase, are reported to be unchanged in rat hippocampus after chronic morphine treatment [35]. We conclude from these data that a modest supersensitivity to β-adrenergic receptor stimulation is expressed in hippocampal CA1 pyramidal neurons after prolonged exposure to morphine and attribute this to a compensatory upregulation in receptors in response to the opiate-induced suppression in central noradrenergic transmission [8,41]. Nonetheless, the present data do not rule out the possibility that an increase in efficiency of receptor interaction with G_s, which mediates β-adrenergic receptor coupling to adenylate cyclase, might also be involved (see for example [15,26]). In fact, the finding in earlier studies by Kuriyama et al. [15] that coupling between β-adrenergic receptors and adenylate cyclase in rat cerebral cortex may be facilitated during the acute phase of withdrawal suggests that alterations in functional responses to β-adrenergic receptor activation might reflect adaptations in postreceptor events.

The subsensitivity and decreased responsiveness to isoproterenol found in pyramidal neurons in slices from morphine-withdrawn animals might result, in part, from a down-regulation in postsynaptic β-adrenergic receptors that occurs as a consequence of withdrawal-induced hyperactivity within the LC/NE system. Several groups have reported that the spontaneous activity of LC neurons is increased after naloxone-precipitated withdrawal in opiate-dependent rats or monkeys [2,3,29,37], and this increase in noradrenergic neuronal activity in rats has a time course identical to that of various withdrawal signs and behaviors [29]. Others have demonstrated that the turnover rate of NE is increased in rat brain during morphine withdrawal [17,32], and in hippocampus this was associated with an increased synaptic release of NE, as measured by in vivo microdialysis [9]. Since the pyramidal neurons constitute a primary target of LC input to the hippocampus [17,28], postsynaptic β-adrenergic receptors would likely undergo desensitization and possible uncoupling from their effector mechanisms due to excessive activation during the acute phase of withdrawal. Such changes might involve covalent modification of the receptor by phosphorylation by β-adrenergic receptor kinase and subsequent internalization of the receptor from the membrane [34].

Notwithstanding this, results from our earlier binding experiments showed hippocampal β-adrenergic receptor density to be reduced only 27% in morphine-dependent rats studied 32 h after withdrawal [22], which is too little to fully account for the changes in the isoproterenol concentration–response curve that accompany withdrawal. Thus, it is conceivable that the changes in β-adrenergic receptor function that accompany withdrawal might involve alterations in post-receptor events in addition to a down-regulation in the number of membrane receptors. It is noteworthy in this regard that neurons in slices from withdrawn rats showed a reduced sensitivity without change in maximal response to the inhibitory effect of forskolin on the slow AHP. The underlying nature of this change seemed most likely to be upstream from the Ca^{2+}-activated slow K^+ conductance, itself (although direct measure of the conductance is needed to confirm this), since the ability of carbachol to reduce the slow AHP was unaltered in neurons from morphine-withdrawn animals. This might involve modifications that affect the activity of adenylate cyclase or cAMP-dependent protein kinase, but precise information on these issues is not available from the present experiments. In future studies, we plan to use a comparable electrophysiological approach to assess the activity of these and other constituent components of the β-adrenergic receptor-coupled adenylate cyclase cascade in hippocampal neurons before and at various times after withdrawal of morphine-dependent animals.

In summary, the present results suggest that the increase in pyramidal neuron sensitivity to β-adrenergic receptor stimulation found in morphine-dependent animals can largely be attributed to changes in the number of membrane receptors.. On the other hand, alterations in post receptor events may contribute as well to the decrease in functional responsiveness to β-adrenergic agonists that occurs after morphine withdrawal. It is possible that the alterations in β-adrenergic receptor system function demonstrated here may not be due to a direct effect of morphine on pyramidal neurons, but could reflect compensatory adaptations to changes in activity within the LC central noradrenergic

system owing to chronic morphine treatment and withdrawal. This hypothesis could be tested by determining whether intracellular responses of neurons to postsynaptic β-adrenergic receptor stimulation are altered in cultured hippocampal neurons after chronic exposure to morphine in vitro in the absence of noradrenergic synaptic input.

Lastly, there is reason to believe that modifications occurring within the hippocampus may play an important role in certain behavioral changes and physiological adaptations that are associated with morphine dependence and tolerance. The hippocampus is known to play an important role in learning and memory and recent evidence has demonstrated a critical involvement of these processes in the development of associative drug tolerance resulting from an interaction between the drug and environment [36].

Acknowledgements

This work was supported by USPHS Grant DA03365 to H.C.M. and USPHS Training Grant DA07268–01 to J.M.A.

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