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Electrophysiological responsiveness to isoproterenol in rat hippocampal slices correlates with changes in β-adrenergic receptor density induced by chronic morphine treatment

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The effects of chronic morphine treatment and morphine withdrawal on β-adrenergic receptor density and electrophysiological responsiveness in rat hippocampus were examined. Chronic treatment of rats with morphine for 14 days resulted in a 19% increase in the number of β-adrenergic receptors in hippocampus, as measured by the binding of the specific antagonist [^{3}H]dihydroalprenolol (DHA). In comparison, the number of specific binding sites for [^{3}H]DHA was decreased 27% in hippocampus in morphine-withdrawn animals, compared to saline-treated controls. These alterations in β-adrenergic receptor density were not accompanied by a significant change in the dissociation constant (K_d) for [^{3}H]DHA or in the inhibitory constants (K_i) for the displacement of the [^{3}H]-antagonist by either norepinephrine or isoproterenol. Electrophysiological experiments in the in vitro hippocampal slice preparation revealed that responses to threshold as well as maximal concentrations of isoproterenol were significantly enhanced in morphine-dependent animals, compared to controls, whereas electrophysiological responsiveness to maximal concentrations of isoproterenol was decreased in slices from morphine-withdrawn rats. The results of this study indicate that β-adrenergic receptors in hippocampus are up-regulated during the development of morphine dependence and down-regulated during opiate withdrawal. These changes in hippocampal β-adrenergic receptor density are likely to be of functional relevance since they are manifested in a corresponding increase and decrease, respectively, in electrophysiological responsiveness to an exogenously administered β-adrenergic receptor agonist.

INTRODUCTION

Several laboratories have reported an increase in the density of β-adrenergic receptors, as measured by the binding of the selective antagonist [^{3}H]dihydroalprenolol ([^{3}H]DHA), in the cerebral cortex in rats after chronic morphine treatment[8,13,22,23]. An increase in the number of cortical β-adrenergic receptors has also been reported after the precipitation of withdrawal in morphine-dependent rats[12]. In the latter case it was shown that the elevation in cortical [^{3}H]DHA binding was due mainly to an increase in the density of β_1-type adrenergic receptors[12]. Studies of adrenergic receptor binding in cerebral cortex after central noradrenergic denervation indicate that the effects of the cortical norepinephrine (NE) input from the locus coeruleus (LC) in rat are mediated via β_1-type adrenergic receptors which, for the most part, are localized postsynaptically on cerebrocortical neurons[19,21]. It would therefore seem to be of some importance to determine whether the alterations in cortical β-adrenergic receptor density that have been reported in morphine-dependent animals before and after opiate withdrawal are of relevance physiologically.

Recently, we confirmed the finding from previous studies that the density of β-adrenergic receptors is increased in the cerebral cortex in morphine-dependent animals, and showed further that these receptors undergo a biphasic change, decreasing significantly in number from control levels 32 h after morphine withdrawal[23]. By way of microiontophoretic testing, it was shown that the shifts in cortical β-adrenergic receptor density which accompanied

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dependence and withdrawal were paralleled by a selective increase and decrease, respectively, in the sensitivity of parietal cortical neurons to direct postsynaptic applications of β-adrenergic agonists, and thus likely to reflect alterations in physiologically relevant populations of postsynaptic neuronal receptors. Functional interpretations of these iontophoretic measures of adrenergic receptor super- and subsensitivity are difficult, however, since quantitative evaluations of dose–response relationships are rarely possible when local application methods of this kind are used to deliver drugs. The interpretation of these electrophysiological data is complicated further due to the fact that the measures of agonist-induced alterations in spontaneous discharge on which those assessments of neuronal chemosensitivity were based have yet to be related in a quantitative manner to the activation of postsynaptic β-adrenergic receptors.

We sought here to overcome some of these difficulties by using the in vitro slice preparation of the rat hippocampus to determine the functional consequences of changes in β-adrenergic receptor content that accompany morphine dependence and withdrawal. In the present experiments, changes in the density and affinity of β-adrenergic receptors in hippocampus after chronic morphine treatment were assessed by measurement of the specific binding of [^{3}H]DHA and compared with alterations in electrophysiological responsiveness to β-adrenergic receptor stimulation in the slice. Electrophysiological assessments of changes in β-adrenergic receptor sensitivity were made by comparing the ability of isoproterenol, applied in known concentrations to the bath, to augment synaptically evoked CA1 population spike responses in slices prepared from control and morphine-treated animals. The results indicate that β-adrenergic receptors in hippocampus are up-regulated during the development of morphine dependence and down-regulated during opiate withdrawal and suggest that these adjustments in receptor density are of relevance functionally, since they are manifested in a corresponding increase and decrease, respectively, in electrophysiological responsiveness of pyramidal neurons to β-adrenergic stimulation.

MATERIALS AND METHODS

Animals and drug treatment

Male Sprague–Dawley rats, weighing 180–220 g at the start of this study, were used. Animals were housed in groups of 6 and allowed free access to food and water. Drugs were obtained from Sigma except when indicated otherwise.

Morphine tolerance and dependence were induced and maintained by giving repeated i.p. injections of morphine sulfate (Mallinckrodt) every 8 h for 14 days. The dosage of narcotic ranged from 10 mg/kg, 3 times a day (t.i.d.), on the first day, being doubled after every third day, to 100 mg/kg, t.i.d., on the last two days. Control animals were given i.p. injections of saline according to the same treatment schedule. At the end of the treatment schedule some rats from the morphine and saline treatment groups were challenged with naloxone 1 mg/kg s.c. to assess the development of dependence on the opiate. These animals were not used in the receptor binding or electrophysiological studies.

Radioligand binding studies

Rats were killed by decapitation either 8 h (these animals constituted the opiate-dependent group) or 32 h (the opiate-withdrawal group) after the last drug injection and their brains removed. The brains were dissected and the hippocampi isolated from 6 rats pooled for each experiment. The hippocampi were homogenized in 5 ml of ice-cold Tris-sucrose buffer (5 mM Tris(hydroxymethyl)aminomethane, 0.25 M sucrose) adjusted to pH 8.0. The homogenates were centrifuged at 1000 *g* for 10 min and the supernatants saved and recentrifuged at 40,000 *g* for 10 min. The crude membrane pellet was washed twice with ice-cold Tris-incubation buffer and recentrifuged for 10 min at 40,000 *g*. The final membrane pellet was resuspended in Tris-incubation buffer which consisted of 50 mM Tris(hydroxymethyl)aminomethane adjusted to pH 8.0.

To measure β-adrenergic receptor density, 1-ml aliquots of the neural membranes were incubated in duplicate for 30 min at 25 °C with various concentrations of [^{3}H]DHA (spec. act., 34.1 Ci/mmol; N.E.N., Boston, MA). Non-specific binding was determined by addition of unlabeled propranolol 10^{-5} M to a second pair of incubates. Specific, i.e.

receptor, binding was defined as the difference between total [^{3}H]DHA bound in the absence and presence of propranolol and ranged from 85.7 ± 2.3% at a ligand concentration of 0.1 nM to 70.9 ± 2.4% at a concentration of 6.4 nM. Incubations were terminated by rapid filtration under vacuum through Whatman GF/C glass fiber filters and by washing with two 10-ml aliquots of Tris-incubation buffer (25 °C). After air drying, the filters were placed in scintillation vials and counted for radioactivity at 35% efficiency.

Saturation experiments were conducted at 9 concentrations of [^{3}H]DHA (0.1–25.6 nM). Preliminary estimates of the maximum number of binding sites (B_{max}) and dissociation constants (K_d) for [^{3}H]DHA were determined by use of the computer program EBDA[18]. Final values were calculated by the nonlinear regression program LIGAND, devised by Munson and Rodbard[25]. For displacement studies, IC_{50}'s were estimated by an iterative curve fitting program described by Parker and Waud[26], and the inhibitory constants (K_i) for NE and isoproterenol were calculated by use of the formula of Cheng and Prusoff[5]. Protein was determined by the method of Lowry et al.[14] using bovine albumin as standard. Results are expressed as fmol of [^{3}H]DHA specifically bound per mg of protein. Values given in the text are means ± S.E.M. of the number of experiments shown in parentheses. Data were assessed statistically by means of two-tailed Student's *t*-test and analysis of variance.

Electrophysiological studies

Transverse slices for in vitro electrophysiological recording were cut at 450 μm on a McIlwain tissue chopper and immediately placed in ice-cold medium consisting of (mM): NaCl, 116.4; KCl, 5.4; $CaCl_2$, 2.5; $MgSO_4$, 1.3; $NaHCO_3$, 26.2; NaH_2PO_4, 1.0; and glucose, 11; which was pregassed with 95% O_2 and 5% CO_2. Slices were transferred within 5 min to an incubation chamber, where they lay on a nylon mesh at the interface of medium (at 27 °C) with an atmosphere of 95% O_2/5% CO_2. After allowing at least 1 h for recovery, slices were transferred individually to a recording chamber and superfused continuously under submersion with medium warmed to 35 °C. A total of 161 slices from 48 rats was examined.

Bipolar stimulating electrodes, fashioned from twisted nichrome wires, were placed under visual guidance in the stratum radiatum near the border of CA1-CA2. Synaptic responses were evoked by stimulating the Schaffer collateral commissural afferents to the CA1 region of the hippocampus at 1 min intervals and recording the evoked population spike responses from the pyramidal cell layer. The responses that were obtained at a given level of stimulus voltage were averaged over 5 trials using a digital oscilloscope (Nicolet model 4094) and stored on diskette for subsequent analysis. Periodically, recordings were also obtained from the region of synaptic termination of the Schaffer collaterals in the dendritic layer, and the amplitude of the evoked field excitatory postsynaptic potential (EPSP) responses measured used to monitor the constancy of pyramidal cell synaptic activation[1,24]. Isoproterenol was found to have no significant effect on the EPSP amplitude in any experiment, which is in agreement with findings from earlier studies[24]. The increases in population spike amplitude produced here by isoproterenol can therefore be interpreted to reflect an increased responsiveness of pyramidal cells to an essentially unchanged excitatory synaptic input.

Monophasic 0.1 ms pulses of 3–45 V were delivered to the slice while testing the synaptic response until potentials of maximum amplitude were obtained from a particular recording site. The intensity of the stimulation was then adjusted to a level at which a population spike of approximately 50% of the maximal response was elicited. Under these conditions, the maximal increases in population spike amplitude that were produced by the β-adrenergic receptor agonist were nearly twice the level of the control response. To assess the effect of isoproterenol on the population spike response, the slice was superfused with medium containing a known concentration of the agonist L-isoproterenol (25–500 nM), and the maximal increase in amplitude of the population spike response obtained in the succeeding 10 min period noted. Most of the slices were tested with at least two and often 3 concentrations of drug. However, because tachyphylaxis and biphasic effects sometimes developed with repeated or prolonged exposure to high concentrations of isoproterenol, a given slice was not exposed to both of the higher doses (250 nM and 500 nM) of the

agonist that were studied.

Data analysis

Responses that were elicited from different slices, prepared from rats of the same treatment group, were combined by expressing the population spike amplitude during superfusion of isoproterenol as a percentage of the predrug response. In this way, the responses from a number of slices superfused with the same concentration(s) of drug could be quantitatively compared, and complete dose–response curves for increases in population spike amplitude by isoproterenol constructed for slices prepared from saline-treated control, morphine-dependent and morphine-withdrawn animals. Differences between mean values were assessed statistically using a paired *t*-test.

RESULTS

Assessment of morphine dependence

The 14-day regimen of chronic morphine administration resulted in a high degree of physical dependence in the treated animals. Physical dependence on morphine was indicated by the abrupt appearance of prominent withdrawal signs (e.g. hyperactivity, spontaneous jumping and diarrhea) shortly after the administration of naloxone or by the occurrence of an abstinence syndrome which began from 10 to 12 h after the cessation of drug treatment in those animals that were withdrawn gradually from the opiate. The rats that were withdrawn from morphine showed an average weight loss which exceeded 28% of total body weight at 32 h of withdrawal, similar to that observed previously[23].

Changes in β-adrenergic receptor binding in hippocampus

Computerized analysis of saturation-binding experiments indicated that [^{3}H]DHA bound to a single population of binding sites in the hippocampal membranes with an apparent K_d of 1.03 ± 0.39 nM (controls, $n = 4$). Specific binding of [^{3}H]DHA was 85 ± 1.4% at ligand concentrations of both 0.8 nM and 1.6 nM which were in the range of the K_d. Fitting of the binding data to a one ligand/one site model yielded Scatchard plots that were essentially linear, and these fits were significantly better than those obtained with a one ligand/two binding site model. The maximum number of specific binding sites (B_{max}) for [^{3}H]DHA in hippocampal membranes from control rats, as determined by the non-linear regression analysis program LIGAND, was 39.8 ± 3.2 fmol/mg protein (Table I).

Chronic treatment of rats with morphine resulted in a significant increase in the binding of [^{3}H]DHA to the hippocampal membranes (Fig. 1). The B_{max} for [^{3}H]DHA (47.3 ± 3.9 fmol/mg protein, $n = 6$) was increased 18.8% ($P < 0.05$) in morphine-dependent rats, when compared to saline-treated controls (Table I, Fig. 1). No significant difference in the K_d for the radioligand was observed between control and morphine-dependent groups.

The increase in hippocampal [^{3}H]DHA binding found with morphine-dependent animals did not occur after acute treatment with morphine (30

TABLE I

Effects of chronic morphine treatment and withdrawal on β-adrenergic receptors in the rat hippocampus

Determinations of specific [^{3}H]DHA binding to hippocampal membranes were made 8 h (chronic morphine) and 32 h (withdrawal) after the last injection of a 14-day chronic morphine treatment schedule. Hippocampi from 6 animals were pooled for each experiment. Values represent the mean ± S.E.M. of the number of experiments shown.

Treatment group	*Number of experiments*	*Parameters of [^{3}H]DHA binding*		*% Change in B_{max}*
		B_{max} (fmol/mg prot.)	*K_d (nM)*	
Control	4	39.8 ± 3.2	1.03 ± 0.39	–
Chronic morphine	6	47.3 ± 3.9*	1.13 ± 0.27	+18.8
32 h-Withdrawal	8	29.2 ± 5.2**	0.70 ± 0.16	−26.6
Acute morphine	3	38.8 ± 3.8	0.98 ± 0.20	−2.8

*$P < 0.05$.

**$P < 0.005$ compared to control values (two-tailed Student's *t*-test).

mg/kg, i.p. at 8 h intervals) for 1 day (Table I). Moreover, morphine added to the incubation medium at a concentration of 10^{-4} M had no appreciable effect on the specific binding of [^{3}H]DHA to hippocampal membranes prepared from control or morphine-treated animals. These data suggest that the increase in number of hippocampal β-adrenergic receptors observed in morphine-dependent animals develops as a direct consequence of prolonged exposure to the opiate.

The effects of opiate withdrawal on hippocampal β-adrenergic receptors were assessed by examining the binding of [^{3}H]DHA to hippocampal neural membranes isolated from chronic morphine-treated rats 32 h after cessation of morphine treatment, by which time the primary abstinence syndrome had abated. Withdrawal of the morphine-dependent animals was accompanied by a decrease in [^{3}H]DHA binding in the hippocampus (Fig. 1). The B_{max} for [^{3}H]DHA in the withdrawn animals was 29.2 ± 5.2 fmol/mg protein (n = 8), which represented a decrease of 26.6% ($P < 0.005$) and 38.3% ($P < 0.001$) in hippocampal β-receptor density, compared to saline-treated controls and morphine-dependent animals, respectively (Table I). The reduction in hippocampal binding sites for [^{3}H]DHA that was found after morphine withdrawal occurred without appreciable change in the affinity of the receptors for the antagonist radioligand (K_d of 0.70 ± 0.16 nM compared to control value of 1.03 ± 0.39 nM, $P > 0.05$) (Table I).

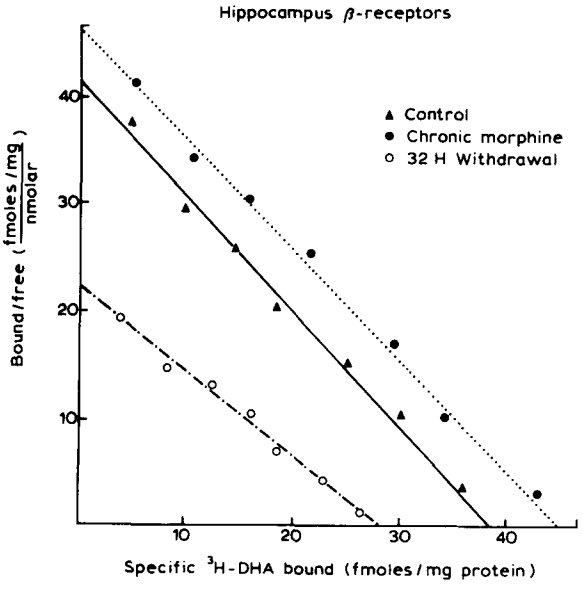

Fig. 1. Scatchard plots showing the effects of chronic morphine treatment (closed circles) and morphine withdrawal (open circles) on the specific binding of [^{3}H]DHA to neural membranes isolated from rat hippocampus. Data are from representative experiments. Points plotted represent determinations, carried out in duplicate, of the binding of [^{3}H]DHA at concentrations of 0.1–25.6 nM to membranes isolated from the hippocampi of 6 animals. Binding affinities, indicated by the slopes of the regression lines drawn through the data points, were not changed after either treatment, whereas the maximum number of binding sites for [^{3}H]DHA (intercept with abscissa) was increased in the hippocampus after chronic morphine treatment and reduced during morphine withdrawal, relative to control. Values for B_{max} and K_d were determined by non-linear regression using the computer program LIGAND.

Since [^{3}H]DHA is an antagonist and changes in β-adrenergic sensitivity and responsiveness in hippocampus were assessed electrophysiologically with agonists, it was also important to determine whether any changes in agonist affinity accompanied morphine dependence or withdrawal. These determinations were made by comparing the ability of isoproterenol and NE to compete with [^{3}H]DHA for β-adrenergic binding sites in membranes prepared from control, chronic morphine-treated and morphine-withdrawn rats. The potencies of isoproterenol and NE in competing for specific [^{3}H]DHA binding sites were determined by incubating a fixed concentration of [^{3}H]DHA (2.6 nM) in the presence or absence of 8 concentrations of competing drugs. These experiments yielded sigmoidal competition curves (Fig. 2) having slope factors somewhat less than unity (0.70–0.85). No significant change was found in the K_i of isoproterenol or NE for displacement of [^{3}H]DHA from hippocampal membranes from rats treated chronically with morphine or after morphine withdrawal (Table II). Moreover, there was no change in the slopes of the displacement curves under these experimental conditions (Fig. 2).

Assessments of electrophysiological responsiveness to isoproterenol

Superfusion of hippocampal slices with the selective β-adrenergic receptor agonist isoproterenol (50–500 nm) increased the amplitude of population spike responses (Fig. 3), in agreement with earlier reports[2,24,30]. The population spike increase reached a maximum within 6 min after beginning perfusion with isoproterenol, and recovery of the spike to the

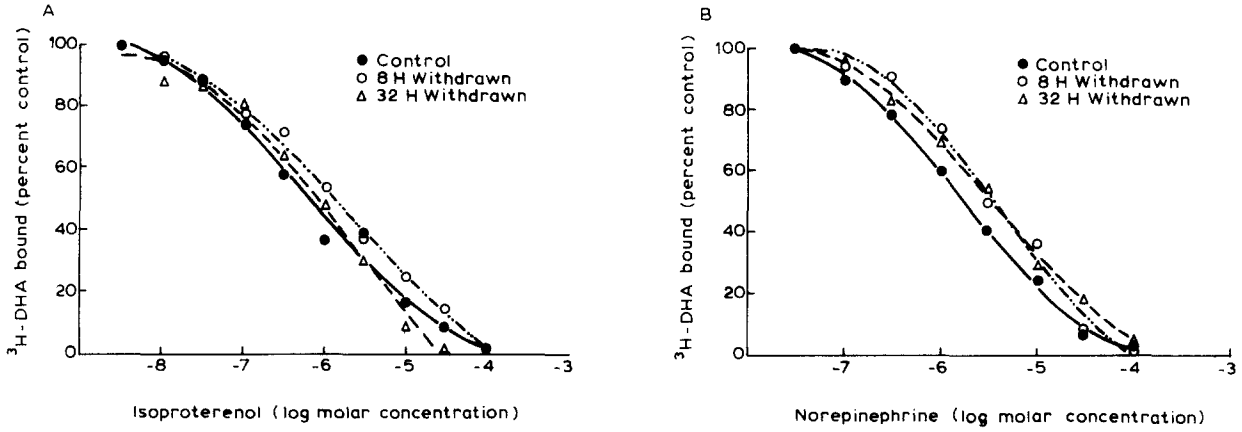

Fig. 2. Inhibition of specific [^{3}H]dihydroalprenolol binding by isoproterenol (A) and norepinephrine (B) in membranes from hippocampus of opiate-naive (filled circles), chronic morphine-treated dependent (open circles), and withdrawn rats (open triangles). Hippocampal membranes, prepared as described in 'Materials and Methods', were incubated with a fixed concentration of [^{3}H]dihydroalprenolol (2.6 nM) in the absence or presence of 8 concentrations of L-isoproterenol or L-norepinephrine. The results are expressed as the percent of [^{3}H]dihydroalprenolol bound in the absence of competing agonist. Results are from typical experiments using hippocampi pooled from 6 animals.

control amplitude was usually obtained within 20–45 min. Those slices in which recovery to the baseline level of the response was not obtained after washout of isoproterenol were excluded from the analysis.

To show that the facilitating effect of isoproterenol was mediated by a β-adrenergic receptor, the ability of the β-antagonist timolol (Merck) and the α-antagonist phentolamine (Ciba-Geigy) to block the enhancement elicited by a single concentration of the agonist (250 nM) was examined. For these

TABLE II

Inhibition constants for norepinephrine and isoproterenol at hippocampal [^{3}H]dihydroalprenolol binding sites in control, chronic morphine-treated and morphine-withdrawn rats

Experiments were carried out using pooled membranes isolated from the hippocampi of 6 rats. Membrane suspensions were incubated in duplicate for 30 min with a fixed concentration of [^{3}H]DHA (2.6 nM) in the presence or absence of various concentrations of NE (100 M–1.0 mM) or ISO (10 nM–0.1 mM). IC_{50} values were calculated by the iterative curve fitting program of Parker and Waud. K_i values were calculated from the IC_{50} values by the method of Cheng and Prusoff. There was no significant effect of chronic morphine treatment or morphine withdrawal on the inhibitory potencies of NE or ISO at [^{3}H]DHA β-adrenergic receptors in hippocampus (two-tailed Student's *t*-test). Each value is the mean ± S.E.M. of 3 experiments.

Adrenergic agonist	*Treatment*	K_i (μM)
Norepinephrine	Control	1.39 ± 0.69
	Chronic morphine	0.68 ± 0.03
	32-h Withdrawal	0.61 ± 0.03
Isoproterenol	Control	0.14 ± 0.07
	Chronic morphine	0.24 ± 0.07
	32-h Withdrawal	0.16 ± 0.06

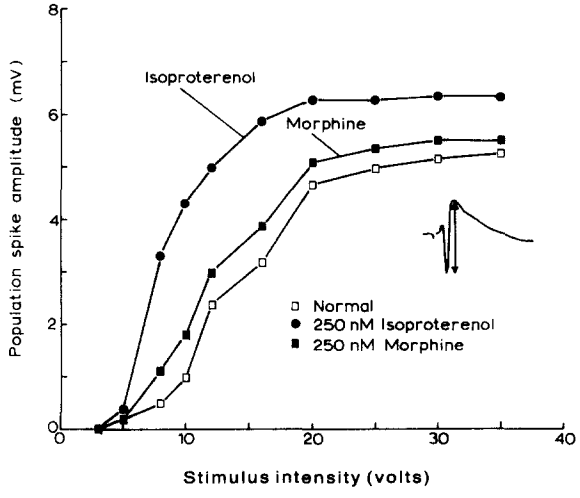

Fig. 3. Input–output curves of the population spike amplitude versus voltage from a single hippocampal slice generated in normal bathing medium (open squares), and during perfusion of 250 nM isoproterenol (solid circles) or 250 nM morphine (solid squares). Effects of isoproterenol or morphine on the population spike response were assessed from 10 to 20 min after start of drug perfusion. Insert shows a typical field potential, which consists of a slow positive wave (upwards) interrupted by a negative–positive population spike, reflecting the synchronous firing of pyramidal neurons in response to Schaffer collateral stimulation. Arrows indicate the measurement used for calculation of the population spike amplitude.

experiments, input–output curves (stimulus voltage versus response amplitude) were constructed in 14 slices from controls by recording synaptic responses over a range of stimulation voltages. In each of the cases, an enhancement of population spike amplitude by isoproterenol was readily discerned by a parallel shift to the left in the input–output curve, as illustrated in Fig. 4. Superfusion of timolol 300 nM completely antagonized the facilitating effect of isoproterenol (6 of 8 slices), and shifted the input–output curve for isoproterenol back to the right toward the baseline response profile (Fig. 4). In comparison, phentolamine at concentrations up to 25 μM did not reduce the response to isoproterenol in any of the 8 slices tested (Fig. 4). Neither antagonist had any direct effects on population spike amplitude within the range of concentrations that were examined.

To compare the facilitating effect of isoproterenol in slices from control and chronic morphine-treated animals, the population spike was adjusted to 50% of maximum amplitude prior to perfusion of the agonist. Isoproterenol was tested at 5 concentrations, ranging from 25–500 nM. Complete dose–response curves for enhancement of population spike amplitude by isoproterenol were constructed by pooling data from many slices taken from controls and from animals of each chronic morphine-treatment group (i.e. 8 h-dependent and 32 h-withdrawn) (Fig. 5). In slices from control rats, the threshold dose of isoproterenol (minimum concentration of agonist required to elicit a 20% increase in the response) was 50 nM, and the effect of the β-adrenergic agonist continued to increase up to a concentration of 500 nM. It was not possible to define a clear maximal response to isoproterenol, however, because higher concentrations of the drug elicited highly variable increases in the spike amplitude and in some cases depressant responses. The effects that were produced by 500 nM isoproterenol were taken, instead, to define the maximal response, since large increases in population spike amplitude were reliably obtained with this concentration of the agonist in slices from both control and chronic morphine-treated animals (Table III).

Chronic treatment of rats with morphine resulted

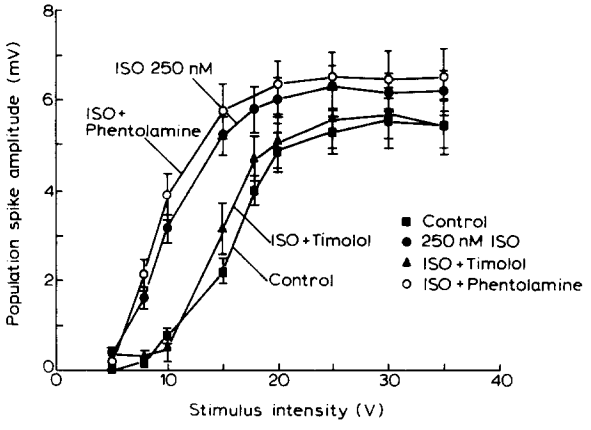

Fig. 4. Effect of α- and β-adrenergic antagonists on the enhancement of population spike amplitude by isoproterenol. Mean population spike input–output curves for control slices generated before (filled squares, n = 14) and during superfusion of 250 nM isoproterenol in the absence (filled circles, n = 14) or presence of the β-antagonist timolol (filled triangles, n = 8) or the α-antagonist phentolamine (open circles, n = 8). Each data point in a curve represents the mean of the averaged evoked response elicited by 5–8 stimuli (0.1 Hz) for 8 (antagonist present) or 14 experiments (no antagonist). Once the initial control input–output curve for a given slice was established the effects of superfusion of 250 nM isoproterenol in the presence and then absence of 300 nM timolol or 25 μM phentolamine were determined. Note the antagonism of the facilitating action of isoproterenol by timolol but not by phentolamine. The standard errors are indicated by the capped bars.

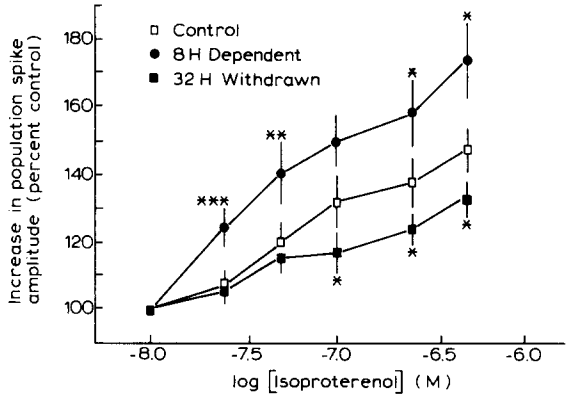

Fig. 5. Sensitivity to isoproterenol as measured electrophysiologically in rat hippocampal slices from saline controls (open squares) and chronic morphine-treated dependent rats before (solid circles) and after withdrawal (32 H, solid squares). Points represent mean values for 11–18 slices from at least 6 animals. Higher concentrations of isoproterenol (>500 nM not shown) elicited variable effects on population spike amplitude in all groups and were therefore excluded from the analysis. Differences between values from control and drug-treatment groups were assessed statistically using a two-tailed Student's t-test with unpaired observations. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$.

in a shift to the left of the dose-response curve for isoproterenol (Fig. 5). This increase in electrophysiological responsiveness in morphine-dependent animals can be seen clearly in Table III, which shows the maximum percentage increase in the population spike produced by isoproterenol in concentrations of 25–500 nM in slices from control, chronic morphine-treated, and morphine-withdrawn rats. Isoproterenol elicited significantly greater increases in population spike amplitude in slices from dependent animals, compared to controls, over the entire range of agonist concentrations tested. The threshold concentration of isoproterenol was reduced to 25 nM ($P < 0.001$, compared to controls) and the maximal responsiveness to the β-adrenergic agonist, measured at 500 nM was increased by 54% ($P < 0.05$) as a result of chronic morphine treatment.

Withdrawal of morphine-dependent rats was accompanied by a decrease in electrophysiological responsiveness to β-adrenergic stimulation in the hippocampus (Fig. 5). Perfusion of isoproterenol in concentrations of 100, 250 and 500 nM elicited significantly ($P < 0.05$) smaller increases in population spike responses in slices from withdrawn animals than controls, with the maximal responsiveness being reduced by 36% 32 h after morphine withdrawal (Table III). In comparison, the threshold dose of isoproterenol for augmenting population spike responses was not significantly different between control and withdrawn groups of slices (Table III).

Morphine has been shown to have an excitatory effect on monosynaptically evoked CA1 field responses of hippocampal slices which is manifested by an increase in the population spike amplitude to Schaffer collateral stimulation near threshold[7,27]. It was therefore possible that the increase in electrophysiological responsiveness to isoproterenol observed in slices from morphine-dependent animals might be due in part to the presence of residual morphine in the hippocampus at the time of sacrifice. To control for this possibility, slices from additional groups of opiate naive ($n = 20$, 4 animals) and morphine-dependent rats ($n = 24$, 6 animals) were maintained in bathing medium containing 250 nM morphine prior to and during the testing of the effects of isoproterenol at 25 nM and 500 nM on population spike amplitude. This concentration of morphine was determined in preliminary experiments (involving both isotopic counting after [^{3}H]morphine injection and high performance liquid chromatography) to be of the order of that found in the hippocampus at the time of sacrifice of our dependent animals, and was also the highest bath concentration of the alkaloid that could be used without initiating acute potentiative effects in slices from controls (see Fig. 3). As shown in Table IV, both concentrations of isoproterenol continued to elicit significantly greater responses in slices from dependent animals than naive controls despite continuous exposure of individual slices from both groups to exogenous morphine (250 nM). These data

TABLE III

Effects of chronic morphine treatment and morphine withdrawal on hippocampal responses to isoproterenol

Responses to isoproterenol are expressed as the mean ± S.E.M. maximum percentage increase in the predrug (baseline) population spike amplitude. The predrug population spike amplitudes were adjusted to be 50% of the maximal response, with values which ranged from 1–4 mV. Numbers in parentheses indicate the number of slices tested at a given concentration. Differences between values from control and drug-treatment groups were assessed statistically using two-way analysis of variance and a two-tailed Student's *t*-test with unpaired observations.

Isoproterenol concentration	*Percent increase in population spike amplitude*				
	Control	*Chronic morphine*	*Significance*	*32-h Withdrawal*	*Significance*
25	7 ± 2 (12)	25 ± 4 (12)	$P < 0.001$	7 ± 2 (12)	NS
50	20 ± 4 (13)	41 ± 7 (14)	$P < 0.05$	16 ± 3 (14)	NS
100	32 ± 8 (13)	50 ± 6 (18)	NS	16 ± 5 (15)	$P < 0.05$
250	39 ± 6 (14)	59 ± 8 (17)	$P < 0.05$	24 ± 4 (16)	$P < 0.05$
500	50 ± 7 (11)	77 ± 10 (12)	$P < 0.05$	32 ± 5 (11)	$P < 0.05$

NS = not significant.

TABLE IV

Enhancement by isoproterenol of population spike responses in slices from naive and chronic morphine-treated rats

Mean values ± S.E.M. Numbers in parentheses indicate the number of slices tested at a given concentration. Differences between values from control slices maintained in normal (morphine-free) medium and values from slices (of control and dependent animals) maintained in medium containing morphine were tested by Student's *t*-test.

	Percent increase in response amplitude by isoproterenol	
	25 nM	*500 nM*
Control slices maintained in normal medium	11 ± 4 (13)	52 ± 5 (12)
Control slices maintained in medium containing morphine (250 nM)	16 ± 6 (18)NS	61 ± 9 (16)NS
Slices from dependent rats maintained in medium containing morphine (250 nM)	28 ± 7 (20)*	82 ± 9 (17)*

*$P < 0.05$.
NS = $P > 0.05$.

argue against a significant contribution of the effects of residual morphine to the increase in β-adrenergic responsiveness found in the hippocampal slices from dependent rats.

DISCUSSION

The results presented here indicate that chronic, but not acute, administration of morphine results in alterations in the density of β-adrenergic receptors in the rat hippocampus. Using an incremental schedule of morphine injections to make animals tolerant and dependent, we observed a 19% increase relative to controls in β-adrenergic receptors when [^{3}H]DHA binding in hippocampus was measured 8 h after cessation of opiate treatment. Animals at this time were judged to still be in a dependent state, owing to the absence of demonstrable withdrawal behaviors or signs. In comparison, the number of binding sites for [^{3}H]DHA was decreased by 27%, relative to saline-treated controls, in hippocampus in dependent animals that were studied 32 h after morphine withdrawal, at a time when the abstinence syndrome had become fully manifested and after prominent withdrawal signs (e.g. diarrhea, hyperactivity, screeching on touch), had begun to abate. These findings are consistent with previous studies in which measurements of [^{3}H]DHA binding in cerebral cortex of morphine-dependent rats revealed an up-regulation and down-regulation of β-adrenergic receptors, respectively, in animals studied before and after undergoing opiate withdrawal[8,13,22,23].

Minneman and Holtzman[20] were unable to detect alterations in β-adrenergic receptor density in several brain areas (i.e. cerebral cortex, cerebellum and striatum) either during long-term induction of morphine dependence or after the manifestation of withdrawal in rats made tolerant and dependent using a 1-month regimen of scheduled access to morphine drinking solutions. The discrepancy in results between studies may be related, in part, to differences in drug treatment regimens, since an incremental dosage schedule of morphine pellet implantations or i.p. injections was used to chronically administer the opiate in all studies where changes in central β-adrenergic receptor density have been observed[8,12,13,23]. This in turn could translate into substantial differences in the degree of tolerance and physical dependence achieved among subjects of these treatment groups. Consistent with this was the finding that weight loss after spontaneous morphine withdrawal, considered to be the most reliable index of the morphine abstinence syndrome in the rat[29], averaged 28% of total body weight in dependent animals in the present study, compared to only 7% in animals rendered dependent by scheduled access to morphine drinking solution[20]. These data suggest that there may be a critical level of dependence required before significant changes in β-adrenergic receptors are induced or otherwise sustained. In addition, they support the idea that the elevations in β-adrenergic density in hippocampus found here and those demonstrated previously in the cerebral cortex after chronic morphine treatment might be related in some way to the formation of the dependent state.

The major issue addressed in these experiments was whether the alterations in β-adrenergic receptor density that occur in hippocampus after chronic morphine treatment or withdrawal are reflected in corresponding changes in postsynaptic sensitivity or responsiveness to β-adrenergic stimulation. We were

able to demonstrate by recording extracellularly in vitro in the hippocampal slice that the up-regulation of β-adrenergic receptors that resulted after chronic morphine treatment was temporally correlated with an enhancement in electrophysiological responsiveness to the β-adrenergic agonist isoproterenol. The enhancement in β-adrenergic electrophysiological responsiveness in slices from morphine-dependent rats was similar to other functional measures of β-receptor supersensitivity[10,13,28,30] in that the magnitude of the increase in the population spike response to either a maximal (54% augmentation at 500 nM) or threshold concentration (355% increase at 25 nM) of isoproterenol was considerably greater than the increase observed in the number of receptors (19%). Zahniser et al.[30] have reported a similar pattern of increased sensitivity of hippocampal slices to isoproterenol after destruction of hippocampal NE innervation by pretreatment with the selective noradrenergic neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DPS4), a treatment which also has been shown to elevate hippocampal β-adrenergic receptors.

Conversely, slices that were taken from morphine-dependent subjects that had undergone withdrawal were found to exhibit a significant decrease, relative to controls, in electrophysiological responsiveness to isoproterenol, at a time when measurements of [^{3}H]DHA binding revealed a substantial decrease in hippocampal β-adrenergic receptor density. This reduction in hippocampal responsiveness to β-adrenergic effects on population spike responses was manifested at higher (i.e. maximal) concentrations of isoproterenol, whereas the increases in response elicited at threshold concentrations of the agonist were not significantly different in slices from morphine-withdrawn rats and opiate-naive controls. The latter findings complement a recent study by Anwyl and Rowan[2], in which similar decreases in electrophysiological responsiveness of hippocampal slices to isoproterenol were demonstrated after chronic treatment of rats with the tricyclic antidepressant imipramine, a procedure known to produce a down-regulation in hippocampal β-adrenergic receptors[3,6].

Intracellular recording studies in the rat hippocampal slice preparation have shown that the activation of postsynaptic β-adrenergic receptors results in blockade of a calcium-dependent potassium conductance and spike frequency accommodation in CA1 pyramidal cells[9,15,16]. It is thought that through this combined action exogenously administered β-adrenergic agonists directly increase the responsiveness of pyramidal cells to depolarizing stimuli and thereby augment the amplitude of synaptically evoked population spike responses that are recorded extracellularly[15,16,24]. Accordingly, the changes in hippocampal electrophysiological responsiveness to isoproterenol demonstrated here after chronic morphine treatment and morphine withdrawal can be interpreted to most likely reflect the development of supersensitivity and subsensitivity, respectively, in postsynaptic β-adrenergic receptors.

Several lines of evidence provide additional support for this possibility. First, the shifts in hippocampal β-adrenergic receptor density that accompanied morphine dependence and withdrawal occurred without change in the affinities of the agonists NE or isoproterenol for the receptors. Second, alterations in transmitter re-uptake were unlikely to contribute in any significant way to the observed functional alterations, since isoproterenol is not a substrate of the NE re-uptake system[4]. Finally, isoproterenol continued to elicit significantly greater β-adrenergic receptor-mediated functional responses in slices from dependent animals than naive controls even when recordings were obtained during superfusion of these preparations with bathing medium containing exogenous morphine (250 nM). This finding suggests that alterations in pyramidal cell excitability resulting from the effects of a residual presence of morphine in the hippocampus of opiate-treated animals were also unlikely to account for the electrophysiological changes reported here. Taken together, these data argue strongly that the changes in hippocampal responsiveness to β-adrenergic stimulation that occur after long-term morphine treatment and withdrawal can be attributed to adaptations involving postsynaptic components of the β-adrenergic receptor system in pyramidal neurons.

The alterations in hippocampal responsiveness to isoproterenol that accompanied morphine dependence and withdrawal need not be related solely to adjustments in the number of β-adrenergic receptors on the pyramidal neurons, but could also reflect postreceptor events which ultimately lead to the

cellular response. Several laboratories have now presented evidence for the involvement of a receptor-coupled adenylate cyclase, cyclic AMP-generating system in the transduction of β-adrenergic electrophysiological actions in both individual pyramidal neurons[15,17] and slices of rat hippocampus[24]. It is therefore conceivable that the amplification in signal transfer provided by the cyclase-cyclic AMP cascade might explain the disparities between the small increase in β-adrenergic receptors and marked enhancement in electrophysiological responsiveness to isoproterenol found in hippocampus in morphine-dependent subjects. In keeping with this idea are earlier findings by Llorens and co-workers[13] who showed in the rat cerebral cortex that the elevations in β-adrenergic receptor density after chronic morphine treatment were accompanied by a much larger increase in isoproterenol-stimulated accumulations of cyclic AMP. The recent report by Kuriyama et al.[11] that the coupling between β-adrenergic receptors and adenylate cyclase in rat cerebral cortex may be facilitated during the acute phase of morphine withdrawal has suggested an additional mechanism whereby the functional expression of β-adrenergic agonist interactions with postsynaptic neuronal receptors might largely be dictated by alterations in postreceptor events. The measures of β-adrenergic receptor response taken here afford little insight regarding the fundamental nature of the perturbation(s) within the postsynaptic receptor-effector system which underlie(s) the alterations in hippocampal electrophysiological responsiveness. Nevertheless, our ability to demonstrate a strong concordance between changes in β-adrenergic receptor binding in hippocampus and related functional alterations in the slice highlight the usefulness of the hippocampal slice preparation as a model system for studying the compensatory response of the β-adrenergic receptor system to long-term opiate administration. Intracellular measures are now being taken from pyramidal neurons in rat hippocampal slices to determine whether the changes in β-adrenergic receptor response found during morphine dependence or withdrawal might be due in part to alterations occurring beyond the receptor level.

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