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Changes in Cortical β-Adrenergic Receptor Density and Neuronal Sensitivity to Norepinephrine Accompany Morphine Dependence and Withdrawal

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Radioligand binding experiments were carried out in conjunction with electrophysiological recordings in vivo in the parietal cortex in rats to assess changes in postsynaptic β-adrenergic receptor function that result after chronic administration of morphine and during morphine withdrawal Chronic treatment of rats with morphine for 14 days resulted in a 38% increase in the density of β-adrenergic receptors in the parietal cortex, as measured by the binding of the specific antagonist [^{3}H]dihydroalprenolol (DHA) In comparison, following withdrawal in the chronic morphine-treated animals, the number of specific [^{3}H]DHA binding sites in this same cortical region was decreased 25%, when compared to saline-treated controls These alterations in cortical 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 specific agonists norepinephrine and isoproterenol Microiontophoretic testing revealed that the changes in β-adrenergic receptor density found in parietal cortex after chronic morphine treatment and during morphine withdrawal were accompanied by a selective increase and decrease, respectively, in the sensitivity of cerebrocortical neurons in the same region to β-adrenergic stimulation These results suggest that changes in central adrenergic function might be related to the formation and/or expression of dependence on morphine

INTRODUCTION

Evidence from both experimental and clinical studies indicates that central noradrenergic mechanisms may play an important role in the formation and/or mediation of opiate withdrawal. For example, depletion of norepinephrine (NE) levels in brain prior to the precipitation of withdrawal has been shown to reduce the intensity of the withdrawal syndrome in morphine-dependent rats[4,10], whereas a marked potentiation of withdrawal intensity has been observed following administration of agents known to augment central noradrenergic activity[10]. In addition, Redmond et al [29] have shown that in subhuman primates direct stimulation of the locus coeruleus (LC) elicits a profile of behavioral and physiological effects strikingly similar to those observed during opiate withdrawal. Both morphine and the α_2 adrenergic agonist clonidine have been shown to block these effects of LC activation[30] and to suppress the acceleration in LC neuron firing and NE turnover in brain that occur during acute opiate withdrawal[1,32] Clonidine has also been shown to alleviate opiate withdrawal symptoms in animals and man[8,30]; an effect which has been attributed in large part to its inhibitory influence on LC noradrenergic neuronal activity These data have led some workers to suggest that increased activity of central noradrenergic neurons, particularly those of the LC, may account for significant components of the opiate withdrawal syndrome

Other data suggest that alterations in adrenergic receptors on LC target cells may contribute to or reinforce the effects occurring in the opiate-sensitive noradrenergic neurons. Several laboratories have reported an increase in β-adrenergic receptor density, as measured by the binding of the selective antagonist [^{3}H]dihydroalprenolol ([^{3}H]DHA), in areas such

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as the cerebral cortex, hippocampus and brainstem in rats treated chronically with morphine[9,17,24]. An increase in the number of β-adrenergic receptors labelled with [^{3}H]DHA has also been observed in the iris of rat pups made dependent on morphine[20]. On the other hand, Kuriyama et al.[15] reported that the number of specific binding sites for [^{3}H]DHA was unchanged in whole brain or cerebral cortex in morphine-dependent rats, but increased abruptly following precipitation of withdrawal in the dependent animals by naloxone. It was further shown that the abrupt increase in [^{3}H]DHA binding found in the cortex during withdrawal was due mainly to an increase in the density of β_1-type adrenergic receptors[13]. Much evidence indicates that the effects of the LC input to rat cortex are mediated via β_1-type adrenergic receptors which, for the most part, are located postsynaptically on cerebrocortical neurons[22,23]. Accordingly, it would be important to determine whether the alterations in cortical β-receptor density that have been reported after chronic morphine treatment or withdrawal result in a corresponding hypersensitivity of these same neuronal elements to noradrenergic stimulation. The functional correlates of the changes in cortical β-adrenergic receptor density that have been observed in both dependent and withdrawn animals have yet to be assessed at the single neuron level.

Neurons within the parietal cortex in rat are known to receive a prominent innervation of noradrenergic fibers from the LC[25,37], and are inhibited by NE acting postsynaptically via a β-adrenergic receptor[2,3,27]. In addition, changes in β-adrenergic receptor density have been reported in this brain region in chronic morphine-treated rats[24]. In the present study, radioligand binding experiments were carried out in the parietal cortex in conjunction with in vivo microiontophoretic testing of drugs to determine whether changes in β-adrenergic receptor density that result from chronic morphine treatment are reflected in corresponding alterations in postsynaptic sensitivity of cerebrocortical neurons to NE. Experiments were carried out at early and late times following withdrawal of chronic morphine treatment to assess the possible relationship of changes in cortical β-adrenergic receptor function to the abstinence syndrome. Some of this work has been reported previously in preliminary form[24].

MATERIALS AND METHODS

Preparation of animals

Male Sprague–Dawley rats, weighing 170–225 g at the start of this study, were used. Animals were housed in groups of 6 and allowed free access to food and water. Sixty-two animals were used in the electrophysiological experiments and more than 120 animals in the binding studies. All animals within the experimental groups received an identical regimen of chronic morphine treatments. However, in order to maintain uniform sampling times following drug treatment and avoid possible confounding effects of anesthesia associated with the electrophysiological testing, different animals were used for the receptor binding and recording experiments. Morphine tolerance and dependence were induced and maintained by giving repeated i.p. injections of morphine sulfate 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 (for the withdrawal group) after the last drug injection and their brains removed. The brains were dissected and the parietal cortices isolated from 6 rats pooled for each experiment. The brain tissues 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 Specific binding was 88.3 ± 1 2% at ligand concentrations of both 0.8 nM and 1 6 nM which were in the range of the K_d. 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[21] Final values were calculated by the non-linear regression program LIGAND, devised by Munson and Rodbard[26] Hill plots were also generated from the computer analysis to determine the presence or absence of receptor cooperativity For displacement studies, IC_{50}'s were estimated by an iterative curve fitting program described by Parker and Waud[28], and the inhibitory constants (K_i) for NE and isoproterenol (ISO) were calculated by use of the formula of Cheng and Prusoff[6] Protein was determined by the method of Lowry et al.[18] using bovine albumin as standard Results are expressed as femtomoles of [^{3}H]DHA specifically bound per milligram 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

Extracellular recording and microiontophoresis

Fifty-four of the animals studied electrophysiologically were maintained under halothane anesthesia. In 8 additional experiments, a decerebrate, unanesthetized preparation was used to control for possible confounding effects of anesthesia on central noradrenergic activity and endogenous opioid function Rats were anesthetized initially with halothane (2.5% in air), intubated and mounted in a stereotaxic frame. The respiratory anesthetic was then either lowered to 0.75% and maintained at that level throughout the experiment or held constant while a surgical transection of the brainstem was performed at a level just anterior to the LC Low cerveau-isolé transections were performed according to procedures described by Wang and Aghajanian[36] using a retractable wire knife. After transection, halothane anesthesia was discontinued to these animals Wound edges and pressure points in both preparations were infiltrated with a solution of 2% procaine hydrochloride (Abbot Labs.) on a regular basis Core temperature of the animals was maintained at 37 °C by means of a servo-control temperature regulating device

A tandem animal protocol, similar to that described by Schultz et al.[34], was employed for testing neuronal sensitivity to microiontophoretically applied agonists. Briefly, a coded control animal and a coded morphine-treated or morphine-withdrawn animal were prepared concurrently and placed in separate stereotaxic frames on the recording table Both animals were usually prepared for recording by someone other than the experimenter in order to carry out the sensitivity testing in a single-blind fashion, minimizing experimenter bias. A midline incision was made in the scalp and a craniotomy performed to expose a circumscribed portion of the parietal cortex, extending 2–5 mm lateral from the midline and 1 mm anterior to 3 mm posterior of bregma (see Fig 2A). After removal of the dura, the exposed pial surface was covered with ungelled agar in balanced salt solution

Five- and seven-barreled glass micropipettes (3–5 μm diameter tips) were used to record extracellularly from single cerebrocortical neurons and to apply drugs at the recording site by microiontophoresis. Recording barrels were filled with 4 M NaCl or a solution of 2 M NaCl saturated with Fast green dye and had in vitro impedances of 3–8 MΩ. Drug barrels were backfilled with freshly prepared solutions of (−)-NE-HCl 0 5 M, pH 4.5 (Sigma); (−)-isoproterenol bitartrate 0.25 M, pH 4 8 (Sigma); (+)-isoproterenol bitartrate 0 25 M, pH 4 8 (Sigma), sotalol hy-

drochloride 0.25 M, pH 4.5 (Regis Chemical); phentolamine mesylate 0.25 M, pH 4.8 (Ciba-Geigy); or γ-aminobutyric acid (GABA) 0.1 M, pH 3.5 (Sigma). Drug solutions were ejected as cations or retained by application of 15 nA currents of opposite polarity Automatic current balancing was maintained through an additional peripheral barrel containing 3 M NaCl. Positive and negative currents were independently passed through this barrel to check for possible current artifacts.

For these experiments, recording of neuronal activity was confined to a uniform 3 × 4 mm area of tissue within the sensorimotor region of the parietal cortex. This same area of cortex comprised a significant portion of the tissue used in biochemical determinations of [^{3}H]DHA binding to cortical β-adrenergic receptors Because this area of cortex contains a heterogeneous population of neurons, cells were first tested for their responsiveness to peripheral tactile stimulation in order to define a relatively uniform population of test neurons. Only those units which responded to brisk mechanical displacements of the hair or glabrous skin of the contralateral fore- or hindlimb were included in this analysis. These were located 200–1200 μm deep to the cortical surface. We have previously demonstrated that NE exerts both a direct depressant action and a GABA-facilitating effect in these cells, mediated via a β-type adrenergic receptor[38]

Action potentials of single cerebrocortical neurons were amplified and displayed on an oscilloscope using conventional electrophysiological techniques. The neural signals were separated from background activity and converted to uniform voltage pulses by a window discriminator. These pulses were integrated over intervals of 1 or 2 s by an electronic ratemeter and the averaged firing rate of each cell displayed on a strip chart recorder to monitor responses to microiontophoretically applied test substances Once a suitable cell was identified (based on the above criteria) and a stable baseline of activity recorded, neuronal sensitivity to β-adrenergic stimulation was assessed by determining the minimal iontophoretic current of NE (or other agonist drug) required to produce a depression in spontaneous firing (see below)

In judging neuronal sensitivity to agonists by iontophoretic methods it is essential to control for the variability in drug release among different pipettes[11]. To compensate for this variability, a strategy based on the high-low threshold method of iontophoretic testing devised by Schultz et al.[34] was used to optimize drug ejection from any one micropipette and then the same micropipette was used to alternately record from cortical cells in tandemly prepared control and experimental animals. Briefly, the threshold iontophoretic NE current required to depress spontaneous firing of a neuron was determined by delivering regularly repeating 20-s pulses of drug (with 60 s between pulses to ensure recovery) at a sufficiently high level to elicit at least a 50% inhibitory response. Drug pulses were then alternated between a similar fixed high effective current, designed to maintain a uniform tip concentration of drug in preparation for the subsequent low 'test' pulse, and a low NE 'test' current which was progressively reduced. With each double pulse cycle, the low NE current was further reduced until a level was reached which just produced an inhibitory response (defined as a 15–20% change from baseline); this current level defining the iontophoretic NE threshold. Changes in neuronal sensitivity to β-adrenergic stimulation after long-term opiate treatment were assessed by comparing the mean iontophoretic current thresholds of NE required to inhibit the firing of cerebrocortical cells recorded in chronic morphine-treated or morphine-withdrawn and chronic saline-treated animals. Differences in mean iontophoretic current thresholds between control and experimental groups of animals were evaluated statistically by means of two-tailed Student's *t*-test and one-way analysis of variance.

RESULTS

Assessment of morphine dependence

The 14-day regimen of chronic morphine administration produced a high degree of physical dependence in the treated animals. Those chronically treated rats in which naloxone was administered displayed a prominent withdrawal syndrome which consisted of hyperactivity, spontaneous jumping, diarrhea accompanied by marked weight loss, teeth-chattering and/or wet-dog shakes. Rats that were withdrawn gradually from morphine displayed an abstinence syndrome of somewhat diminished intensity (i.e. no jumping), with weight loss at 32 h of withdrawal that typically exceeded 25% of total body weight

In these latter animals, the earliest signs of withdrawal routinely appeared 10–12 h following the cessation of morphine treatment Similar changes in behavior and loss in body weight were not observed in either non-withdrawn experimental or saline-treated control animals.

Changes in cortical β-adrenergic receptor binding

High affinity binding of [^{3}H]DHA to the cortical neural membranes was rapid, saturable and reversible by propranolol 10^{-5} M Computerized analysis of saturation binding experiments indicated a single population of binding sites for [^{3}H]DHA with an apparent K_d of 0.87 ± 0.09 nM (controls, n = 5) The fits obtained with a one ligand/one site model yielded Scatchard plots that were essentially linear, and these 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, as determined by the non-linear regression analysis program LIGAND, was 62.3 ± 3 3 fmol/mg protein in the parietal cortex of control animals. These B_{max} and K_d values for [^{3}H]DHA binding are in good agreement with those reported in rat cortex by other workers[15,17]

Chronic administration of morphine resulted in a significant increase in the binding of [^{3}H]DHA in the parietal cortex (Fig 1). The B_{max} for [^{3}H]DHA (86 1 ± 3.7 fmol/mg protein, n = 8) was increased 38.2% ($P < 0.002$) in morphine-dependent rats, when compared to saline-treated controls (Table I, Fig. 1) This increase in density of cortical β-adrenergic binding sites occurred without a significant change in K_d for the radioligand (Table I) In addition, the Hill

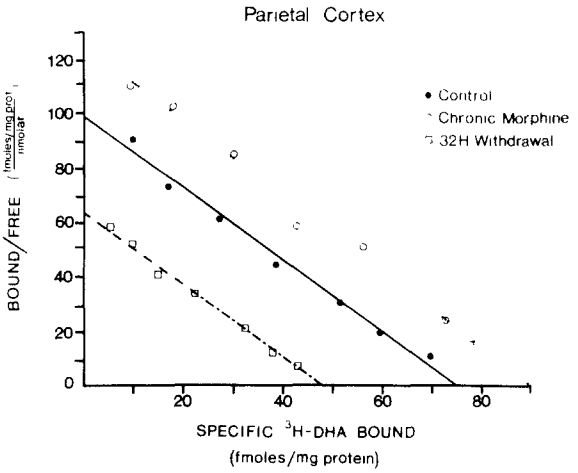

Fig 1 Scatchard plots showing the effects of chronic morphine treatment (open circles) and morphine withdrawal (open squares) on the specific binding of [^{3}H]DHA to neural membranes isolated from rat parietal cortex Data are from representative experiments Points plotted represent determinations, carried out in duplicate, of the binding of [^{3}H]DHA at concentrations of 0 1–25 6 nM to membranes isolated from the cortices 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 ordinate) was increased in the cortex after chronic morphine treatment and reduced following morphine withdrawal, relative to control Values for B_{max} and K_d were determined by non-linear regression using the computer program LIGAND

number for [^{3}H]DHA binding in the parietal cortex in morphine-dependent rats was unchanged compared to that for the saline-control group (Table I) These data strongly suggest that changes in the properties of the β-adrenergic receptors, themselves, are unlikely to account for the elevation in [^{3}H]DHA binding found in the cortex after chronic morphine treatment.

TABLE I

Effects of chronic morphine treatment and withdrawal on the specific binding of [^{3}H]dihydroalprenolol to rat cortical membranes

Determinations of specific [^{3}H]DHA binding to the cortical membranes were made 8 h (chronic morphine) and 32 h (withdrawn) after the last injection of a 14-day chronic morphine treatment schedule Cortices 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 binding*			*% Change in B_{max}*
		B_{max} *(fmol/mg prot)*	K_d *(nM)*	n_H	
Control	5	62 3 ± 3.3	0 87 ± 0 09	1 03 ± 0 03	
Chronic morphine	8	86 1 ± 3 7*	1 14 ± 0 15	1 04 ± 0 03	+38 2
32-h withdrawn	9	46 5 ± 1.7**	0 69 ± 0 09	1 06 ± 0 01	−25 4
Acute morphine	3	63 2 ± 4.5	0 88 ± 0 20	0 98 ± 0 02	+ 1 4

*$P < 0.002$, **$P < 0$ 001, compared to control values by Student's t-test (two-tailed)

The increase in [^{3}H]DHA binding observed after chronic administration of morphine did not occur after acute treatment of rats with morphine (30 mg/kg, i.p at 8-h intervals) for one day. In these acute experiments, specific binding of [^{3}H]DHA to the cortical membranes was determined at concentrations of radioligand from 1 to 25.6 nM, 8 h after the last of 3 morphine injections. The B_{max} (63.2 ± 4 5 fmol/mg protein, n = 3) and K_d (0.88 ± 0.20 nM) for [^{3}H]DHA binding to membranes isolated from rats treated acutely with morphine did not differ significantly from corresponding values obtained for cortical [^{3}H]DHA binding in the control group (Table I). Moreover, inclusion of up to 10^{-4} M morphine in the incubation assay had no effect on the specific binding of [^{3}H]DHA. Thus, the elevation in cortical β-adrenergic binding sites observed in morphine-dependent animals appeared to emerge as a direct consequence of prolonged exposure to the opiate.

The effects of opiate withdrawal on cortical β-adrenergic receptors were assessed by examining the binding of [^{3}H]DHA to cortical neural membranes isolated from chronic morphine-treated rats 32 h after administration of the last morphine injection. By this time the primary abstinence syndrome had become fully developed and in most animals the 'dominant' withdrawal signs had already begun to subside. Withdrawal of the morphine-dependent subjects was accompanied by a decrease, relative to controls, in [^{3}H]DHA binding in the parietal cortex (Fig. 1) The B_{max} for [^{3}H]DHA in cortex in the withdrawn animals, as determined by computer analysis, was 46.5 ± 1 7 fmol/mg protein (n = 9), which represented a decrease of 25.4% ($P < 0.001$) and 46.0% ($P < 0.001$) in cortical β-receptor density, respectively, compared to saline-treated controls and morphine-dependent animals (Table I). The K_d (0.69 ± 0.09 nM) and Hill number for [^{3}H]DHA binding to cortical membranes isolated from morphine-withdrawn rats, however, were not significantly different from values obtained for control animals (Table I).

Because the functional correlates of these changes in β-antagonist binding were assessed physiologically in relation to the sensitivity of cerebrocortical neurons to NE agonists, it was important to also determine the effects of long-term morphine treatment on agonist affinity at cortical β-adrenergic receptors. These determinations were made by comparing the ability of the β-receptor agonists, ISO and NE, to compete with [^{3}H]DHA for β-adrenergic binding sites on cortical membranes prepared from control, chronic morphine-treated and morphine-withdrawn rats. The potencies of ISO 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 drug. The results of these experiments (Table II) indicated no significant change in the K_i of ISO and NE against [^{3}H]DHA in the cortex after chronic morphine administration or morphine withdrawal. Moreover, there was no change in the slope of the competition curves after these experimental conditions (data not shown)

TABLE II

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

Experiments were carried out using pooled membranes isolated from the cortices 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 nM–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[28] K_i values were calculated from the IC_{50} values by the method of Cheng and Prusoff[6] 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 receptor sites in cortex (two-tailed Student's t-test) Each value is the mean ± S E M. of 3 experiments

Drug	*Treatment*	K_i *(μM)*
NE	Control	2 72 ± 0 48
	Chronic morphine	2.47 ± 0 83
	Withdrawn	1 36 ± 0 47
ISO	Control	0 27 ± 0 15
	Chronic morphine	0 31 ± 0 09
	Withdrawn	0 19 ± 0 06

Electrophysiological assessments of neuronal sensitivity to iontophoretically applied agonists

A total of 272 cerebrocortical neurons were studied for their responsiveness to β-adrenergic receptor stimulation. Forty-six of these neurons were recorded in morphine-dependent rats, 51 cells in morphine-withdrawn animals and 27 cells in animals treated acutely with morphine, with the remainder (n = 148) comprising neurons recorded in matched sa-

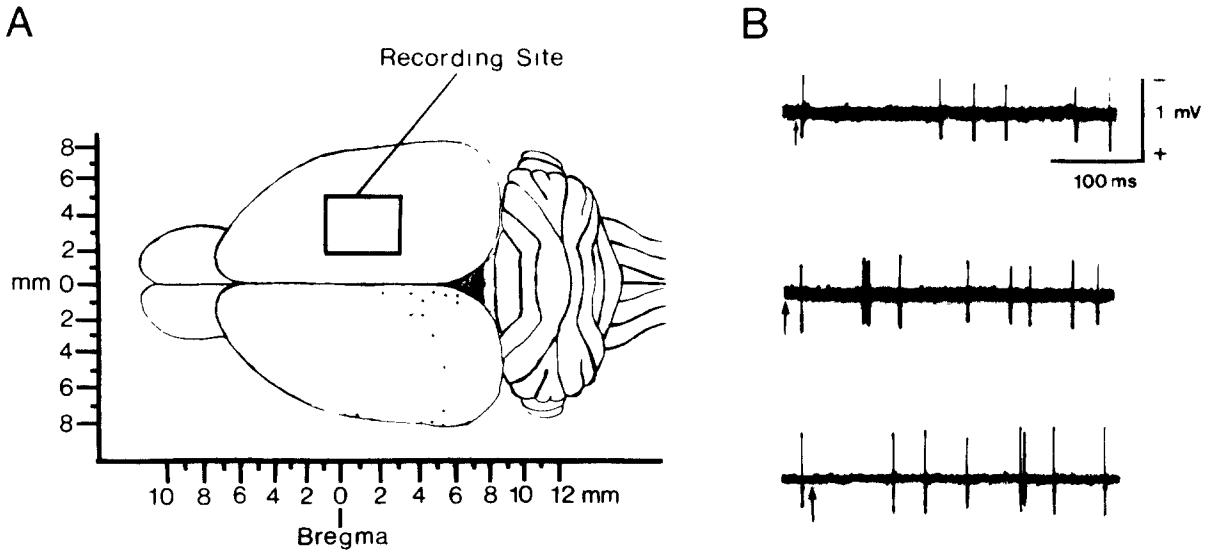

Fig 2 Recording site and responses of somatosensory cortical neurons to tactile stimulation of the contralateral footpad A dorsal view of rat brain showing the area of the cortex (boxed-in area within right hemisphere) from which neuronal recordings were obtained Testing of chemosensitivity of cerebrocortical neurons to iontophoretically applied drugs was carried out in both hemispheres The stippled area over the left hemisphere indicates the region of cortical tissue (obtained bilaterally) used in the radioligand binding experiments B oscilloscope records show the extracellularly recorded responses of cortical neurons to tactile stimulation of the contralateral forelimb (upper and lower traces) and hindlimb (middle trace) which were used to characterize cells prior to iontophoretic testing Stimulus onset is indicated by the arrow in each record Note the difference in latency of the excitatory response between the neurons depicted in the upper and middle traces (see text for further details)

line-treated controls Fig. 2 shows oscilloscope recordings of neuronal responses to tactile stimulation of the fore- and hindlimb which were used as criteria for identifying a relatively homogeneous population of cerebrocortical cells. The typical response elicited by the tactile stimulation consisted of a brief excitation (one to 3 spikes) at 6–31 ms latency, followed by a short period of inhibition (duration to 180 ms), however, excitations alone (21% of cases) or phases of pure inhibition (15% of cases) were observed in some neurons (Fig. 2) Although cerveau-isolé transection precluded the elicitation of this peripherally evoked activity in cortical units, the neurons (n = 24) recorded in the transected animals (n = 8, 4 controls and 4 morphine-treated) displayed rates and patterns of spontaneous discharge remarkably similar to those of neurons recorded in halothane-anesthetized rats. Moreover, no significant differences were noted in chemosensitivity to locally applied drugs between neurons in cerveau-isolé and anesthetized animals which had been obtained from the same treatment group. Results obtained in anesthetized and cerveau-isolé preparations have, therefore, been grouped together according to the respective treatment group (i e. saline or chronic morphine) from which they came

The ratemeter records in Fig 3 illustrate the 'high-low' threshold determination, using the same micropipette, of minimal effective iontophoretic currents of NE, (−)-isoproterenol ((−)-ISO) and GABA required to inhibit the spontaneous firing of single cortical neurons in saline (record A) and chronic morphine-treated rats (record B). Fig. 4 shows the results of similar determinations of iontophoretic thresholds of these drugs required for the inhibition of neuronal firing in a morphine-withdrawn animal (record B), compared to a control rat (record A) In order to correlate these kinds of data with the results of radioligand binding, assessments of changes in neuronal chemosensitivity resulting from chronic morphine administration were carried out from 8 to 12 h following the last injection of the treatment regimen Similarly, determinations of chemosensitivity of cortical neurons in withdrawn rats were confined to a period extending from 32 to 36 h after the cessation of chronic morphine treatment

Iontophoresis of 20-s pulses of NE in the range of 10 to 65 nA produced an inhibition in the sponta-

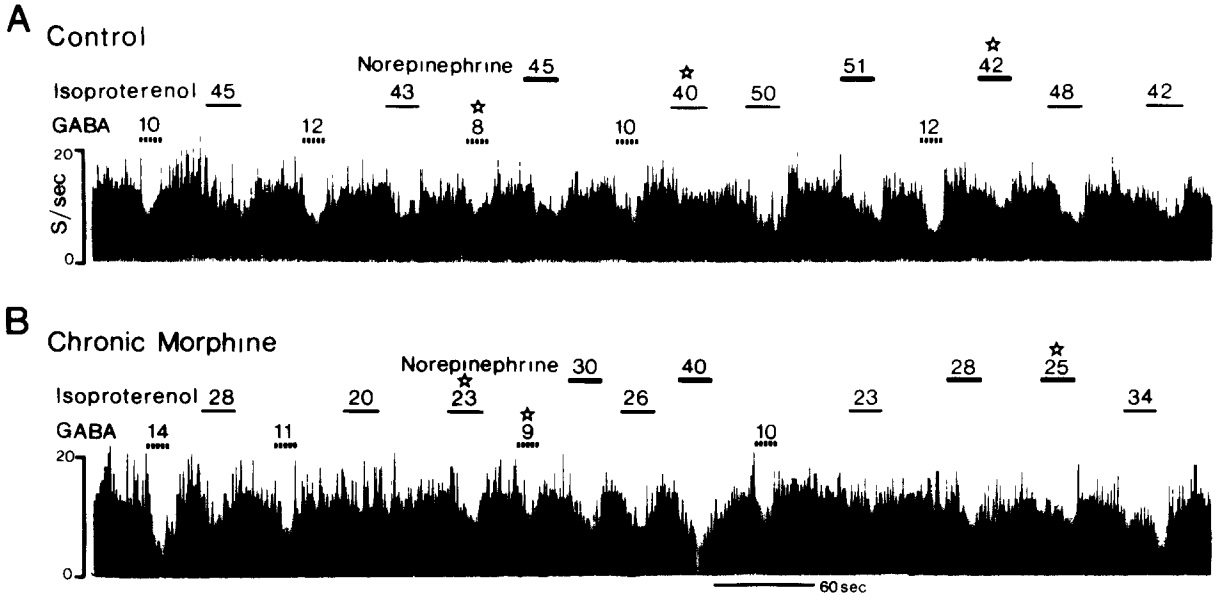

Fig. 3 Ratemeter records from a representative experiment demonstrating use of the high-low threshold method for testing sensitivity to iontophoretically applied transmitter substances in cerebrocortical neurons from a control (A) and chronic morphine-treated animal (B). Assessments of chemosensitivity to iontophoretically applied NE (thick bars), (−)-ISO (thin bars) and GABA (dashed lines) were carried out in tandem between neurons in control and morphine-treated rats, using the same micropipette Short bars above the ratemeter records indicate time of drug application and numbers above the bars refer to ejection current in nanoamperes Note that the cell tested in the chronic morphine-treated animal (B) shows higher sensitivity to the direct inhibitory actions of NE (threshold, 25 nA) and (−)-ISO (threshold, 23 nA) than the control cell (A) (thresholds of 42 and 40 nA, respectively), but that both neurons demonstrate similar chemosensitivity to GABA (thresholds of 8 and 9 nA) The minimal iontophoretic dose of drug required for inhibition of spontaneous discharge of the neuron is indicated by a star above the corresponding ejection current. Assessments of chemosensitivity of neurons in chronic morphine-treated animals were conducted between 8 and 12 h following cessation of chronic drug treatment

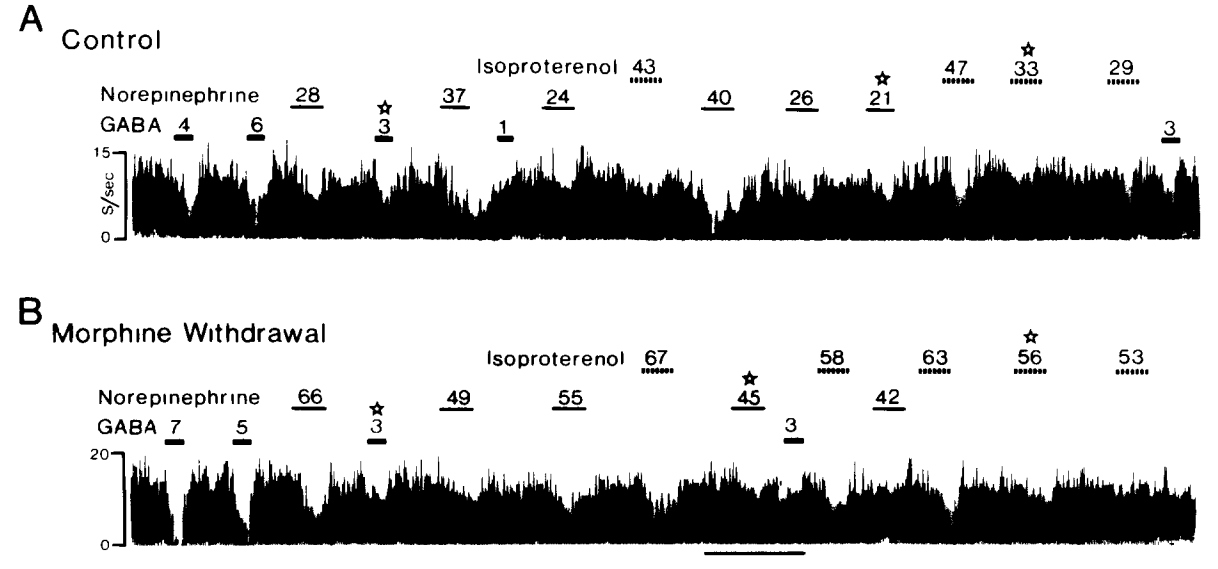

Fig 4 Continuous ratemeter records from an experiment comparing sensitivity to iontophoretically applied transmitter substances of cerebrocortical neurons in a control (A) and a morphine-withdrawn rat (B). The neuron recorded in the withdrawn animal displayed much lower sensitivity to iontophoretically applied NE (thin bar; threshold, 45 nA) and (−)-ISO (dashed line, threshold, 56 nA) than the control cell (thresholds of 21 and 33 nA for NE and ISO, respectively) In comparison chemosensitivity to the inhibitory action of GABA (thick bar) was the same (threshold, 3 nA) for both neurons Chemosensitivity of neurons in control and withdrawn animals to each of the iontophoretically applied drugs was assessed using the same micropipette All tests in withdrawn animals were conducted between 32 and 36 h following cessation of chronic morphine treatment Calibration line under record B indicates 60 s Other details as in legend to Fig 3

TABLE III

Changes in cerebrocortical neuron sensitivity to iontophoretically applied transmitter substances following chronic morphine treatment

Assessments of neuronal chemosensitivity to iontophoretically applied substances were made 8–12 h following the last injection of a 14-day morphine treatment regimen. Adrenergic agents were applied as 20-s pulses and GABA as a 10-s pulse.

Agent	*Cell pairs*	*Mean threshold iontophoretic current*[a]			*Spontaneous firing (Hz)*[b]	
		Controls	*Morphine-treated*		*Controls*	*Morphine-treated*
(−)-NE	46	41.5 ± 1.9	27.3 ± 1.4	$P < 0.001$	14.1 ± 0.9	13.9 ± 1.1
(−)-ISO	37	31.8 ± 1.5	17.9 ± 2.4	$P < 0.001$	12.9 ± 1.5	11.4 ± 1.6
(+)-ISO	17	46.1 ± 2.1	44.5 ± 6.5	n.s.	16.3 ± 2.4	11.9 ± 1.9
GABA	37	5.9 ± 0.8	6.9 ± 1.0	n.s.	13.5 ± 1.0	14.3 ± 1.3

[a] Data are expressed as the mean (± S.E.M.) threshold dose of drug (nA) required to produce an inhibition of spontaneous neuronal firing.

[b] The spontaneous firing rates (expressed as mean ± S.E.M.) of cells recorded in control and chronic morphine-treated rats were not significantly different ($P > 0.25$, one-way analysis of variance).

neous firing of virtually all cortical neurons recorded in control animals. The spontaneous discharge of cortical neurons in both chronic morphine-treated and morphine-withdrawn animals was, for the most part, also readily depressed by brief (20-s) iontophoretic applications of NE (Figs. 3 and 4). In order to assess the β-adrenergic mediation of these depressant noradrenergic effects, inhibitory responses to NE were examined before and during concurrent iontophoresis of the selective antagonists sotalol and phentolamine in 10 neurons from each treatment group. A specific blockade of NE-induced inhibitions in firing was reliably produced by application of the β-antagonist sotalol (22 of 30 cells), but not the α-antagonist phentolamine (3 of 30 cells) in cells in control, chronic morphine-treated and morphine-withdrawn animals (data not shown).

Neurons recorded both in chronically morphine-treated animals and in morphine-withdrawn rats showed marked differences in sensitivity to iontophoretically applied NE, when compared to cells tested in saline-treated controls. Tables III and IV give the mean iontophoretic currents required for inhibition of cortical neurons by various drugs in control, chronic morphine-treated and morphine-withdrawn rats. As shown in Table III, chronic administration of morphine resulted in a significant reduction (from 41.5 ± 1.9 to 27.3 ± 1.4 nA, $P < 0.001$) in the mean threshold iontophoretic dose of NE required to inhibit cortical neuron firing. These findings imply an increased sensitivity of neurons to NE in chronic morphine-treated rats. In contrast, the mean threshold NE current required to depress neurons in morphine-withdrawn rats (45.9 ± 2.1 nA) was significantly ($P < 0.001$) greater than the mean threshold for control cells (23.0 ± 1.4 nA) (Table IV), indicating a reduced sensitivity to NE following withdrawal. The tables also show that despite individual varia-

TABLE IV

Changes in sensitivity of cerebrocortical neurons to iontophoretically applied transmitter substances following morphine withdrawal

Assessments of chemosensitivity of individual neurons to iontophoretic applications of adrenergic agonists (20-s pulses) and GABA (10-s pulses) were made 32–36 h following the cessation of chronic morphine treatment.

Agent	*Cell pairs*	*Mean threshold iontophoretic current*[a]			*Spontaneous firing (Hz)*[b]	
		Controls	*Morphine-withdrawn*		*Controls*	*Morphine-withdrawn*
(−)-NE	51	23.0 ± 1.4	45.9 ± 2.1	$P < 0.001$	17.1 ± 0.9	16.9 ± 0.9
(−)-ISO	44	30.7 ± 1.6	49.2 ± 2.4	$P < 0.001$	16.2 ± 1.1	14.8 ± 1.3
GABA	51	5.3 ± 0.6	6.3 ± 1.2	n.s.	17.1 ± 0.9	16.0 ± 0.9

[a] Data are expressed as the mean (± S.E.M.) threshold dose of drug (nA) required to produce an inhibition of spontaneous neuronal firing.

[b] The spontaneous firing rates (expressed as mean ± S.E.M.) of cells recorded in control and morphine-withdrawn animals were not significantly different ($P < 0.5$, one-way analysis of variance).

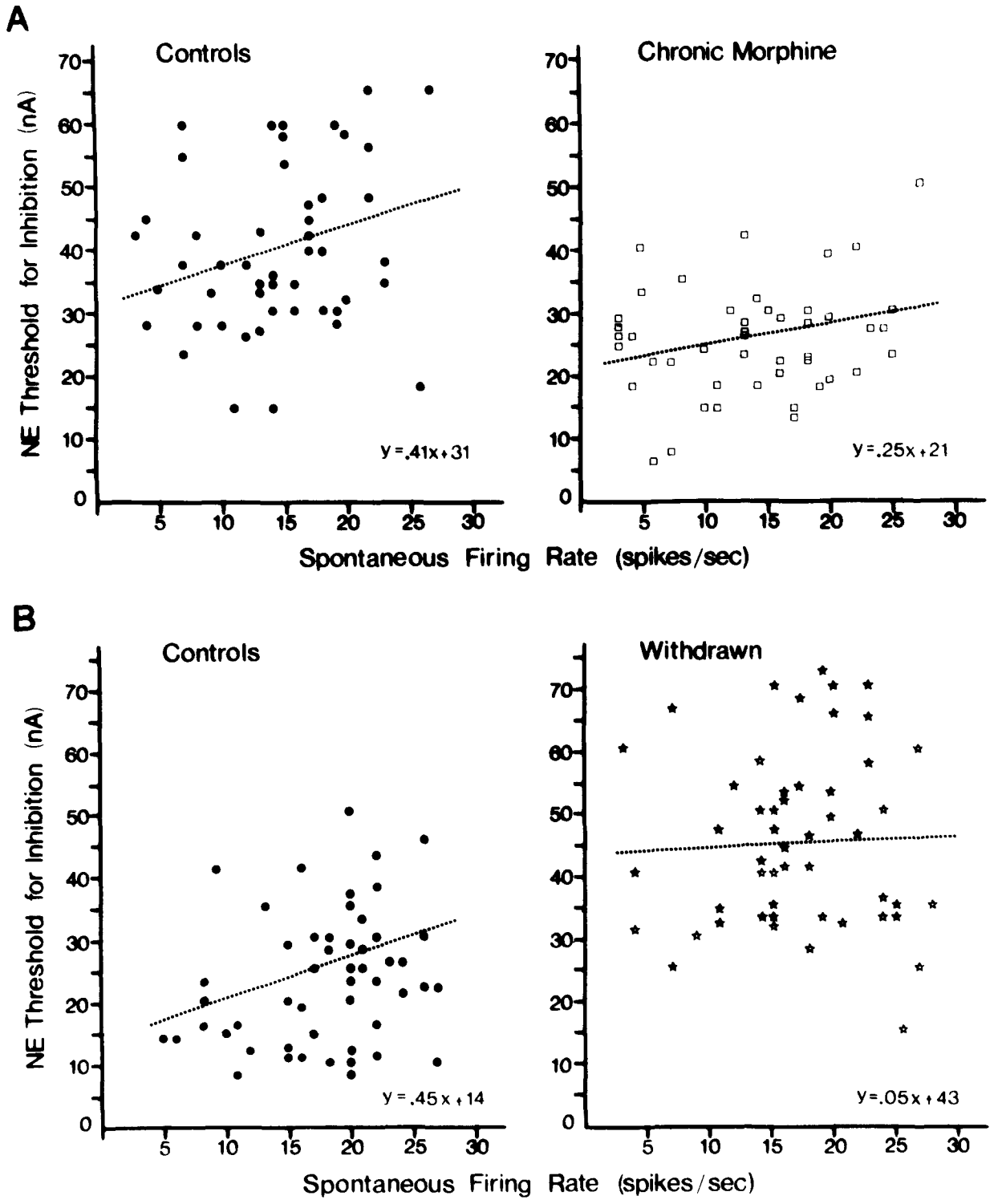

Fig. 5. Scatter plots show little evidence of correlation between sensitivity to iontophoretically applied NE and rate of spontaneous discharge of neurons in control (A and B, left panels), chronic morphine-treated (A, right panel) and morphine-withdrawn animals (B, right panel) Each point plotted represents data from a different neuron Regression analysis of the data from each of the 4 groups yielded the sensitivity/rate lines shown with correlation coefficients of $r = 0\ 19$, $P = 0.19$ for chronic morphine controls; $r = 0.20$, $P = 0\ 18$ for chronic morphine-treated cells; $r = 0.25$, $P = 0\ 08$ for withdrawn controls; and $r = 0.04$, $P = 0\ 78$ for withdrawn neurons A· the slopes of the sensitivity/rate lines for control and experimental groups were compared using the test of Zerbe et al [39] to control for a possible biasing of the results due to the presence of rate as a co-variant. This analysis showed cortical neurons from chronic morphine-treated rats to be significantly more sensitive to NE than control cells ($P < 0.01$) B neurons recorded in morphine-withdrawn animals were found in turn to be significantly less sensitive to NE than cells in matched controls ($P < 0\ 005$) The controls for the chronic morphine-treated and -withdrawn groups consisted of entirely separate populations of cerebrocortical neurons

tions, the mean rates of spontaneous firing of cortical units recorded in chronic morphine-treated and in morphine-withdrawn animals did not differ significantly from that of matched saline controls (Tables III and IV). No correlation was found between the initial rate of spontaneous discharge of a neuron and the minimal effective iontophoretic dose of NE required to inhibit firing in either control ($r = 0.19$, $P = 0.19$ for chronic group; $r = 0.25$, $P = 0.08$ for withdrawn group), chronic morphine-treated ($r = 0.20$, $P = 0.18$) or withdrawn animals ($r = 0.04$, $P = 0.78$) (Fig. 5). Moreover, when the data were re-examined controlling for initial firing rate of the cell as a possible covariant, neurons in chronic morphine-treated and withdrawn rats were still found to be significantly more ($P < 0.01$) and less sensitive ($P < 0.005$) to NE, respectively, than control cells (Fig. 5).

It should be noted that the assessments of changes in cortical neuron sensitivity to NE in morphine-dependent and morphine-withdrawn rats were conducted several months apart, and that different groups of saline-treated animals served as matched controls in each series of experiments. Cortical neurons recorded in the two groups of control animals displayed a considerable difference in sensitivity to iontophoretically applied NE (but not ISO, cf. Tables III and IV). This was most likely related to inherent differences in catecholamine release between the micropipettes used in each of the studies, rather than an indication of considerable variability in the normal range of NE chemosensitivity of the cerebrocortical neurons. In this regard, no significant difference in mean iontophoretic inhibitory thresholds of NE was found in experiments ($n = 3$) in which the same micropipette was used to assess chemosensitivity in matched-pairs ($n = 12$) of cerebrocortical neurons recorded in tandemly prepared saline-treated control rats.

Changes in cellular excitability have been reported during morphine dependence and withdrawal[12,33] which, by themselves, might affect the postsynaptic sensitivity of neurons to exogenously applied catecholamines. Therefore, to control for the specificity of the changes in NE thresholds found among cortical neurons in chronic morphine-treated and -withdrawn rats, iontophoretic current thresholds of GABA required for inhibition of firing were also determined for most cells (Figs. 3 and 4). No significant differences (all P values > 0.1) in the mean current thresholds for inhibition by GABA were found between neurons in chronically morphine-treated and morphine-withdrawn animals, when compared to control cells (Tables III and IV).

The specificity of the increase in noradrenergic sensitivity found in neurons in morphine-dependent animals was assessed further by examining the effects of prolonged morphine treatment on cortical neuron responsiveness to iontophoresis of (+)-isoproterenol ((+)-ISO), an adrenergic agent which has been shown in other test systems to be largely inactive in stimulating β-receptors[16,20]. The effects of iontophoresis of (+)-ISO on cortical unit activity were examined on 25 pairs of neurons recorded in 6 control and 6 chronic morphine-treated rats. Pulsatile applications of (+)-ISO of 20 s duration depressed the ongoing activity of 38 of these neurons (20 control and 18 treated cells); however, these effects required a much higher range of iontophoretic doses (30–75 nA, 47.2 ± 6 nA, mean ± S.E.M.) than was needed to produce inhibitory responses with the full β-receptor agonist, (−)-ISO (see below). Changes in sensitivity of cortical neurons to (+)-ISO following chronic morphine administration were assessed by comparing the minimal effective iontophoretic currents of drug required to inhibit the firing of cells recorded in control and morphine-dependent rats. In experiments performed on 17 tandemly studied cell pairs, no significant difference ($P > 0.25$) was found in sensitivity to (+)-ISO between neurons in control and chronic morphine-treated animals (Table III). These findings, when considered in conjunction with the results of experiments with GABA, argue against the possibility that a non-specific change in cellular excitability accounted for the alterations in noradrenergic sensitivity found in cortical neurons in morphine-dependent and morphine-withdrawn animals.

In order to differentiate the involvement of pre- versus postsynaptic components in the changes in noradrenergic sensitivity found following chronic morphine treatment and withdrawal, analogous experiments were conducted using (−)-ISO which is not a substrate for the high affinity NE reuptake system[5]. The sensitivity to (−)-ISO of neurons in both morphine-dependent (37 cells in 12 rats) and morphine-withdrawn animals (44 cells in 12 rats) differed significantly ($P < 0.001$) from that of control cells (Figs. 3

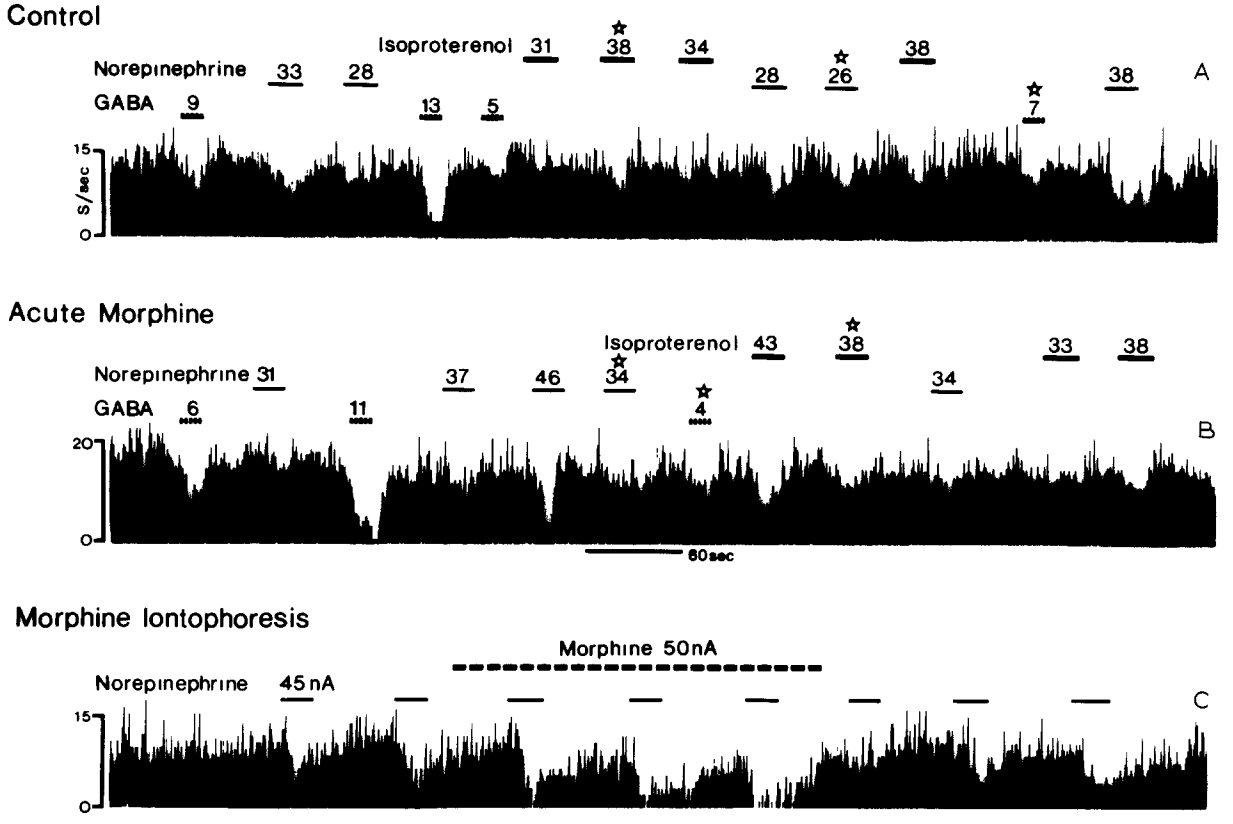

Fig. 6. Effects of acute and local administration of morphine on the sensitivity of cerebrocortical neurons to iontophoretically applied transmitter substances Acute treatment of rats with morphine, 30 mg/kg t.i.d., for one day (B) did not significantly alter cortical neuron responses to iontophoretically appied NE (thin bar), (−)-ISO (thick bar) or GABA (dashed line), relative to the actions produced by these substances in control cells (A). In these experiments, the same micropipette was used to assess chemosensitivity of cortical neurons recorded in control (A) and acute morphine-treated rats. Other details as in legend to Fig 3 C continuous ratemeter record showing inhibitory responses of a cortical neuron in a naive rat to iontophoretically applied NE (45 nA, thin line) before, during and after concurrent iontophoresis of morphine (50 nA, dashed line) onto the cell During morphine iontophoresis spontaneous discharge of the cell was considerably depressed, whereas the inhibitory response to NE showed little appreciable change The apparent increase in NE-induced inhibition observed 3 min after onset of morphine iontophoresis can be attributed to a simple summation of depressant effects produced by both agents.

and 4). Whereas chronic morphine treatment resulted in a marked reduction in the iontophoretic current threshold for inhibition by (−)-ISO (17.9 ± 2.4 nA, mean ± S.E.M.; 8 to 28 nA, range), significantly higher iontophoretic currents of the agonist were required to inhibit the firing of cortical neurons in withdrawn animals (49.2 ± 2.4 nA, mean ± S.E.M.; 23 to 65 nA, range), compared to control cells (14 to 50 nA, range) (Tables III and IV). In all cases, chemosensitivity to GABA was also determined for each neuron, providing a control for assessing receptor specificity. No significant differences (all P values > 0.1) were found between the mean GABA thresholds required to inhibit firing of neurons in chronic morphine-treated and -withdrawn animals, when compared to control cells (Tables III and IV).

In a final set of experiments, an identical type of analysis was carried out to examine the possibility that the presence of residual levels of morphine in brain might contribute to the increase in noradrenergic sensitivity found in cortical neurons after chronic morphine administration. For these acute studies, determinations of the sensitivity of neurons to iontophoretically applied transmitters were carried out in tandemly prepared control and morphine-treated rats 8 h following administration of the last of 3 morphine (30 mg/kg, i.p at 8-h intervals) or saline injections. The upper and middle records in Fig. 6 show the results of a typical experiment. A comparison of these ratemeter records reveals no obvious differences in

TABLE V

Changes in sensitivity of cerebrocortical neurons to iontophoretically applied transmitter substances following one-day morphine treatment

Rats were given 3 i p injections of morphine (30 mg/kg) or saline at 8-h intervals for one day Eight hours after administering the last injection, assessments were made of the sensitivity of neurons in these animals to iontophoretically applied adrenergic agonists and GABA

Agent	*Cell pairs*	*Mean threshold iontophoretic current*[a]			*Spontaneous firing (Hz)*[b]	
		Controls	*Morphine-treated*		*Controls*	*Morphine-treated*
(−)-NE	27	27 4 ± 2 4	33 1 ± 2 7	n s	12 2 ± 1 3	12 4 ± 1 7
(−)-ISO	22	35 1 ± 2 2	39 8 ± 3 3	n s	10 9 ± 1 7	12 8 ± 1 5
GABA	25	6 1 ± 0 9	6 8 ± 1 2	n s	12 4 ± 1 3	12 4 ± 1 7

[a] Data are expressed as the mean (± S E M) threshold dose of drug (nA) required to produce an inhibition of spontaneous neuronal firing

[b] The spontaneous firing rates (expressed as mean ± S E M) of cells recorded in saline-treated control and morphine-treated rats were not significantly different ($P > 0.5$, one-way analysis of variance)

responsiveness of the neuron in the control (Fig. 6A) and acute morphine-treated rat (Fig 6B) to noradrenergic or GABAergic stimulation Overall, no significant differences (all P values > 0.25) were found in the mean iontophoretic inhibitory thresholds for NE, (−)-ISO or GABA between cerebrocortical neurons recorded in control and in acute morphine-treated animals (Table V). In addition, local application of morphine directly onto cerebrocortical neurons had little appreciable effect on postsynaptic depressions in cell firing produced by NE iontophoresis (Fig. 6C) in either control (8 neurons in 3 rats) or acute morphine-treated rats (7 cells in 4 rats)

DISCUSSION

The results of this study confirm and extend earlier findings[9,17] that chronic, but not acute, administration of morphine results in an increased density of β-adrenergic receptors in rat cerebral cortex. Using an incremental schedule of morphine injections to make animals tolerant and dependent, we observed a 38 2% increase, relative to control, in the B_{max} for [^{3}H]DHA in the parietal cortex in chronic morphine-treated rats. This increase in β-adrenergic receptor density was measured 8 h following cessation of opiate treatment when animals were judged to still be in a dependent state, owing to the absence of demonstrable withdrawal behavior or signs In comparison, when binding of [^{3}H]DHA was carried out 32 h after administration of the last morphine injection, a significant decrease (25.4%) in β-adrenergic receptors was found in the parietal cortex in morphine-withdrawn rats, compared to saline-treated controls. These alterations in the density of [^{3}H]DHA-labelled β-adrenergic receptors occurred without significant change in either the K_d or Hill number for the antagonist, suggesting that the character (intrinsic properties) of the β-adrenergic receptors found in the cortex in morphine-dependent and morphine-withdrawn rats may be identical to the native binding sites for [^{3}H]DHA Consistent with this interpretation is the finding that the potencies of the β-adrenergic agonists, NE and ISO, in competing for specific [^{3}H]DHA binding sites in the parietal cortex were also unchanged following chronic morphine treatment or withdrawal.

The time course of the elevation in β-adrenergic receptor density found in the parietal cortex after chronic morphine treatment suggests that the up-regulation of these receptors may be related in some way to the formation of the dependent state. Supporting this possibility are the findings of Hamburg and Tallman[9], who reported a progressive increase in [^{3}H]DHA binding in the cortex with increased time of exposure to morphine in rats implanted with morphine pellets for up to 3 days It was further shown that the increase in cortical β-adrenergic receptor density observed after chronic morphine treatment could be completely reversed by abruptly withdrawing the dependent animals with naloxone 1 h prior to sacrifice The addition of naloxone in vitro did not affect the binding of [^{3}H]DHA to cortical membranes isolated from either control or chronic morphine-

treated animals, leading these workers to suggest that the effects produced by administration of the antagonist in vivo could most likely be attributed to a precipitation of withdrawal (in the morphine-dependent subjects) and the increase in central noradrenergic activity which accompanies it[1,30,32]. Conceivably, such a withdrawal-induced activation of central noradrenergic pathways, leading to an increased postsynaptic availability of NE, could account in similar fashion for the down-regulation in β-adrenergic receptors observed here in the parietal cortex in the withdrawn animals.

A primary objective of the present study was to determine whether the changes in β-adrenergic receptor density that result after chronic morphine treatment or withdrawal are reflected in corresponding alterations in the sensitivity of individual cortical LC target cells to noradrenergic stimulation. Using a high-low threshold method of iontophoretic testing to assess the responsivity of neurons to postsynaptically applied transmitters, we were able to demonstrate that the increase in β-adrenergic receptor density that occurred in the parietal cortex after chronic morphine treatment was temporally correlated with an increased neuronal sensitivity to NE within the same brain area. Conversely, neurons that were tested in animals that had undergone withdrawal from morphine were found to exhibit a subsensitivity to noradrenergic stimulation, compared to cells in controls, at a time when measurements of [^{3}H]DHA binding revealed that the density of β-adrenergic receptors in that region of the cortex was substantially reduced. All of the cells in which noradrenergic sensitivity was assessed were characterized initially as mechanoreceptive units and responded to tactile input originating from either the contralateral forelimb or hindlimb. Previous studies in this[38] and other laboratories[2,3,27] have indicated that the inhibitory effects of NE on spike discharge in these neurons are mediated directly via the activation of postsynaptic β-adrenergic receptors. It seems likely, therefore, that the changes in β-adrenergic receptor density that were revealed through alterations in [^{3}H]antagonist binding to some extent involve these populations of postsynaptic neuronal receptors and may thus be of physiological relevance.

Several lines of evidence suggest that the changes in noradrenergic responsiveness observed in neurons in chronic morphine-treated and -withdrawn rats reflect the development of supersensitivity and subsensitivity in postsynaptic β-adrenergic receptors, respectively. First, the changes in electrophysiological responsiveness to NE that accompanied morphine dependence and withdrawal occurred without alterations in the chemosensitivity of cortical neurons to the inhibitory effects of GABA or of (+)-ISO, an adrenergic agonist which is largely inactive in stimulating β-adrenergic receptors[16,20]. Second, these changes in noradrenergic responses of neurons in chronic morphine-treated and -withdrawn animals were reproduced in tests with (−)-ISO, a β-selective agonist which is not a substrate for the NE reuptake system[5]. Alterations in the neuronal reuptake of NE[7] or a non-specific change in cellular excitability[12,33] are thus unlikely to account for the changes in noradrenergic sensitivity found in cortical neurons following long-term morphine treatment or withdrawal. Finally, local application of morphine directly onto cortical cells did not appreciably alter NE-induced inhibitions in firing elicited in neurons of either control or experimental animals. This suggests that alterations in NE activity at neuronal receptors resulting from a residual presence of morphine in the brain are also unlikely to account for the electrophysiological changes reported here. Taken together, these data make it likely that the changes in neuronal responsiveness to β-adrenergic stimulation that occur after chronic morphine treatment and during withdrawal reflect adaptations or adjustments involving postsynaptic components of the adrenergic system residing in the neurons.

The finding that the changes in cortical β-adrenergic receptor density observed during morphine dependence and withdrawal were accompanied by corresponding alterations in electrophysiological responsiveness to NE does not, by itself, establish a causal relationship between the changes in receptor number and the alterations in noradrenergic response of the effector cells. Clearly, these changes in the responsiveness of neurons to NE need not be related solely to adjustments in the number of β-adrenergic receptors residing on the postsynaptic membrane, but could also reflect postreceptor events which ultimately lead to the electrophysiological response. Some studies suggest that the inhibitory effects of NE on cortical neurons may be mediated via

the coupling of β-receptors to adenylate cyclase[35], and an increase in ISO-stimulated cyclic AMP accumulation has been reported to accompany elevations in β-adrenergic receptor density in rat cortex following chronic morphine treatment[17]. It should be noted that this enhanced responsiveness of the cyclic AMP system to ISO exceeded the increase observed in receptor density, suggesting the likelihood of compensatory changes beyond a simple increase in the number of β-adrenergic binding sites. Interestingly, Kuriyama et al [14] have recently reported that the coupling between β-adrenergic receptors and adenylate cyclase in rat cerebral cortex may be facilitated during acute withdrawal. The functional tests used here to assess changes in noradrenergic responsiveness in cortical neurons provide little insight regarding the locus of change in the postsynaptic adrenergic system which underlies the electrophysiological changes Nevertheless, the demonstration here of a strong concordance between the changes in radioligand binding and electrophysiological responsiveness to NE observed among chronic morphine-treated and -withdrawn animals can be interpreted to support some form of linkage between the alterations in cortical β-adrenergic receptor density and in noradrenergic responsitivity that emerge during morphine dependence and withdrawal.

It is presently unclear to what extent alterations in β-adrenergic receptor density in the brain might contribute either in the formation or expression of dependence on morphine In some studies where increases in cortical β-adrenergic receptor density have been reported in rats treated chronically with morphine, the up-regulation in receptors was noted only after the precipitation of withdrawal in the dependent animals[13,15] In contrast, the results of the present study demonstrate clearly that β-adrenergic receptors are increased in the cortex prior to the onset of withdrawal in morphine-dependent animals and down-regulated during later stages of abstinence at a time when the withdrawal syndrome has become fully manifested This discrepancy in results between these studies may be related, in part, to differences in the timing of receptor binding following spontaneous and naloxone-precipitated withdrawal of the chronic morphine-treated animals One possibility is that while the elevation observed in cortical β-adrenergic receptors may be related to events occurring during dependence, ultimately this up-regulation in postsynaptic receptors could contribute in the formation and/or occurrence of the withdrawal syndrome. The emergence of supersensitivity in central β-adrenergic receptors is unlikely, however, to be primarily responsible for the sympathetic hyperactivity which characterizes the primary abstinence syndrome, since the administration of β-adrenergic receptor blockers, such as propranolol, does not appreciably reduce withdrawal signs in human addicts[31] A second possibility is that the alterations in β-adrenergic receptor density that result after long-term morphine treatment might reflect compensatory adaptations of these postsynaptic systems to changes in turnover rate of NE in the brain (and hence, transmitter availability) which are known to accompany opiate withdrawal[30,32] In this regard, the down-regulation in cortical β-adrenergic receptors that was found in the dependent animals during later stages of abstinence could result from the central noradrenergic hyperactivity which occurs during the acute withdrawal phase in both rats and primates[30,32] If the receptor down-regulation found in the cortex were to prove indicative of a subsensitivity in postsynaptic β-adrenergic receptors in LC terminal fields throughout the neuraxis, such a change in noradrenergic responsiveness could account, in part, for the signs of noradrenergic hypoactivity which comprise the 'secondary' or protracted abstinence syndrome observed clinically[19] Studies are currently underway in this laboratory to investigate this possibility

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