glycosylase [18–20], enhanced the formation of (FUra)-DNA. A similar 3- to 5-fold increase in (Ura)DNA formation occurs when human lymphoblast uracil-DNA glycosylase is inhibited by uracil [23]. Although uracil treatment may result in higher intracellular levels of FUra containing nucleotides and thus greater incorporation into DNA, the addition of similar concentrations of uracil in a previous study had no effect on intracellular nucleotide pools [23]. Further, uracil did not enhance incorporation of FUra into RNA.

Uracil-DNA glycosylase initiates a process of excision and repair by producing an apyrimidinic site following removal of FUra [24]. This excision could result in DNA fragmentation. A recent study has demonstrated that treatment with FdUrd results in small size DNA fragments as analyzed by alkaline sucrose gradient centrifugation [25]. This finding could be secondary to excision of Fura residues or to the inhibition of DNA synthesis by this agent. It should now be possible to employ uracil to inhibit the excision of FUra residues and determine whether there is an associated decrease in DNA fragmentation. It would also be of interest to determine whether uracil prevents the cytotoxicity associated with incorporation of FUra residues in DNA [15].

In summary, recent works [13] has demonstrated that FdUrd misincorporates into MCF-7 human breast carcinoma DNA. The incorporated FUra residues are partially excised from MCF-7 DNA [14] and this may contribute to the cytotoxicity associated with (FUra)DNA formation [15]. We have attempted to extend these findings by monitoring the effect of uracil on the extent of FUra misincorporation into DNA. The results demonstrate that uracil enhances the formation of MCF-7 (FUra)DNA.

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Morphine dependence and withdrawal without alterations in cerebral β-adrenergic receptor density*

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Opiate analgesics appear to have direct effects on catecholamine-containing neurons. Morphine decreases norepinephrine release from sympathetic neurons in the cat nictitating membrane [1] and in the mouse vas deferens [2]. In the brain, opiates decrease the firing of noradrenergic neurons in the locus coeruleus [3], increase catecholamine synthesis [4, 5], and decrease the depolarization-induced release of norepinephrine from cerebellar cortex [6]. Since clonidine, a selective α_2-adrenergic receptor agonist, can partially suppress morphine withdrawal [7], it has been suggested that alterations in the activity of noradrenergic neurons might play a role in physical dependence on morphine. Similarities in the electrophysiological actions of clonidine and morphine have been noted [8, 9], and chronic morphine administration has been shown to increase the apparent number of [^{3}H]

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clonidine binding sites in rat cerebral cortex and brain stem [10].

Other evidence suggests that cerebral β-adrenergic receptors may be involved in morphine tolerance and dependence. An increase in β-adrenergic receptor density and isoproterenol-stimulated adenylate cyclase activity has been reported to be associated with morphine dependence in rat cerebral cortex, cerebellum, brain stem, iris [10–12] and monkey cerebellum [13], although other authors have observed no change in β-adrenergic receptor density in cerebral cortex or brain stem of morphine-dependent rats [14–16]. Withdrawal of chronic morphine administration has been reported to decrease β-adrenergic receptor stimulated adenylate cyclase activity in cerebella from morphine-dependent monkeys [13], and to decrease β-adrenergic receptor density in rat cerebral cortex [14, 15]. It has been suggested that alterations in β-adrenergic receptor density may play a role in some of the phenomena observed when physically dependent animals are withdrawn from morphine [11].

Most forms of chronic morphine administration involve increasingly high dose pellet implantations or injections given over a short period of time (3–10 days), procedures which may be associated with significant stress to the animals, nutritional deficits, and general debilitation. Cerebral β-adrenergic receptor density in rats can be decreased by chronic restraint stress [17, 18], and it was of interest to determine whether β-adrenergic receptor density is altered in the brains of rats exposed to a longer and more gradual procedure for inducing morphine tolerance and dependence. Gellert and Holtzman [19] have described a method for inducing morphine tolerance and dependence in rats by scheduled access to morphine drinking solutions. In this way animals can be maintained in a tolerant/dependent state for months with none of the adverse effects associated with repeated pellet implantations. We here report that there are no changes in β-adrenergic receptor density in three areas of rat brain or in rat heart following scheduled access to morphine drinking solutions for 1 month. Similarly, there is no effect of withdrawal from morphine on β-adrenergic receptor density in these tissues.

Materials and methods

Male Sprague–Dawley rats (100–120 g at the start of the study) were housed singly in cages in a cabinet designed to control the access of each animal to its drinking solution [19]. Food was available *ad lib.* Morphine tolerance and dependence were induced and maintained by scheduled access to 0.05% morphine drinking solutions. This regimen results in a daily intake of approximately 40 mg/kg of morphine in four divided doses [19, 20]. Control animals received scheduled access to water. After a 1-month regimen of scheduled access to morphine, rats were randomly separated into two groups. Half were maintained on an oral morphine schedule ("morphine-dependent") and half were withdrawn from morphine and given continuous access to water ("morphine-withdrawn"). Withdrawn animals were killed 49–52 hr after the last access to morphine, at a time when withdrawal symptoms are of maximal severity.

To measure β-adrenergic receptor density, animals were killed, and the hearts and brains were quickly removed, weighed, and placed in cold 20 mM sodium–Hepes* buffer (pH 7.4) containing 154 mM NaCl ("Hepes–salt"). Cerebral cortex, cerebellum and corpus striatum were dissected from each brain and each tissue was homogenized in 20 ml of Hepes–salt buffer, centrifuged at 20,000 *g* for 10 min, and resuspended in 200 (heart and cerebellum) or 400 (cortex and striatum) vol. of Hepes–salt buffer.

β-Adrenergic receptor density was measured in each tissue by Scatchard analysis of the specific binding of [^{125}I] iodocyanopindolol (ICYP) [21]. Briefly, 100 μl of tissue homogenate was incubated with various concentrations of ICYP (3,000–90,000 cpm; 5–150 pM) in a final volume of 250 μl containing 20 mM sodium–Hepes (pH 7.4), 154 mM NaCl, 100 μM GTP, 1.1 mM ascorbic acid and 1 mg/100 ml bovine serum albumin in the presence or absence of 50 μM 1-isoproterenol. Samples were incubated for 120 min at 37°, diluted with 10 ml of 10 mM Tris–Cl (pH 7.4), and filtered over glass fiber filters (S + S #30) under reduced pressure. Filters were washed with 10 ml buffer, and radioactivity was determined.

Receptor binding of ICYP was defined as the difference between total ICYP binding and ICYP binding in the presence of 50 μM 1-isoproterenol, and was routinely 80–90% of total binding. Saturation isotherms were constructed for six concentrations of ICYP in each tissue, and the data were analyzed by Scatchard analysis, resulting in consistently linear plots. Under these conditions, ICYP binds equally well to both β_1 and β_2 receptors in cerebral cortex with a k_1 of 1.1×10^7 l/mole-sec, a k_{-1} of 1.7×10^{-4} sec^{-1}, and a K_D of about 10 pM. Similar results were obtained in the other tissues.

Protein was determined by the method of Bradford [22] using bovine serum albumin as a standard.

Each experimental group contained five to six animals, and the experiment was repeated twice.

Results

To demonstrate that the animals were physically dependent on morphine, weight loss was monitored during the 48 hr following morphine withdrawal. Loss of body weight is perhaps the most reliable single index of the morphine withdrawal syndrome in the rat [19, 23]. The rats that were withdrawn from morphine showed a 7.2 ± 0.8% (mean ± S.E.M., N = 11) loss in weight over the 48-hr period following withdrawal from morphine, similar to that observed previously [19]. Nonwithdrawn and control animals showed a 3.3 ± 0.8 and a 3.2 ± 0.4% *increase* in body weight, respectively, over the same period of time.

No changes were observed in the density of β-adrenergic receptors in any of the tissues studied. Table 1 is a compilation of two separate experiments. There were no significant differences between β-adrenergic receptor density in control, dependent, or withdrawn animals in cerebral cortex, cerebellum, corpus striatum or heart in either of the two separate experiments, or in the pooled data from both experiments (Table 1). The precision of the measurements, as indicated by the small standard errors in Table 1 (3–6%), suggests that even small changes in receptor density should have been apparent if they had occurred.

Discussion

These experiments demonstrate that long-term morphine dependence and withdrawal can occur without alterations in β-adrenergic receptor density in the brain or in the heart. Although many investigators using other, and shorter, treatment regimens have found alterations in β-adrenergic receptor density in brain and peripheral tissues during chronic morphine treatment [10–13] and withdrawal [13–15], the data presented here suggest that it is unlikely that alterations in β-adrenergic receptor density play a role in the manifestations of morphine dependence and withdrawal. In support of this, other reports have demonstrated no change in brain β-adrenergic receptor density during chronic morphine treatment [14–16]. It seems probable that the differences between these studies lie in the regimen for inducing and maintaining morphine dependence. Possibly the induction of dependence by the rapid high-dose injection or pellet implantation methods produces a greater degree of dependence which is associated with alterations in cerebral β-adrenergic receptor density, or that rapid changes occurring during the first week of morphine

* Hepes = 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

Table 1. Effect of morphine dependence and withdrawal on β-adrenergic receptor density in rat heart, cerebral cortex, cerebellum and corpus striatum*

	B_{max} (fmoles ICYP bound/mg protein)		
	Control	Morphine-dependent	Morphine-withdrawn
Cerebral cortex	149 ± 5.3	140 ± 5.2 (94%)	148 ± 8.0 (99%)
Cerebellum	79 ± 4.4	79 ± 4.8 (100%)	80 ± 3.7 (101%)
Corpus striatum	154 ± 7.3	152 ± 9.7 (99%)	144 ± 9.4 (94%)
Heart	42 ± 1.3	41 ± 1.5 (98%)	44 ± 1.7 (105%)

* Animals were made dependent on morphine by scheduled access to morphine drinking solutions for 1 month. Control animals received scheduled access to water. Morphine-withdrawn animals were withdrawn from scheduled access to morphine 48 hr before being killed. Tissues were collected and prepared, and the density of β-adrenergic receptors was determined by Scatchard analysis of specific ICYP binding as described in the text. Each value is the mean ± S.E.M. for eleven animals, determined in two separate experiments.

administration might be compensated for when morphine is administered for a longer period of time. The reported changes in β-adrenergic receptor density might also represent species differences, or might possibly be related to the stressful nature of some of the procedures for inducing morphine tolerance and dependence.

There do exist well documented alterations in the functioning of adrenergic neurons in the brain during chronic opiate administration, and during tolerance to and dependence on opiates [3–6, 8]. Although we have shown here that these alterations in activity do not alter β-adrenergic receptor density in brain, this does not necessarily imply that there is no change in the functioning of the adrenergic input to these receptors. In some situations, such as acute desensitization of cultured cell lines, β-adrenergic receptors can be "uncoupled" from adenylate cyclase in a manner such that occupation of the receptor by agonist does not result in a physiological response, with no change in the density of β-adrenergic receptors [24]. However, this type of uncoupling occurs only during short-term acute exposure to hormone, and incubation of these cells with hormone for a longer time period will result in a decrease in receptor density proportional to the decrease in responsiveness [24]. As a general rule, long-term hyperstimulation of β-adrenergic receptors is usually associated with decreases in receptor density [25]. Our results suggest that alterations in β-adrenergic receptor density in the brain do not occur during long-term induction of morphine dependence or during the manifestations of morphine withdrawal. It is not yet clear to what extent alterations in the functioning of adrenergic neurons contribute to these phenomena.

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