Selective down-regulation of hippocampal glucocorticoid receptors during opiate withdrawal

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Abstract

The effects of opiate dependence and antagonist-precipitated withdrawal on glucocorticoid (GR) and mineralocorticoid (MR) receptor mRNA levels in the rat brain were studied. Rats were allocated to one of four groups that differed in terms of type of drug pretreatment (morphine pellet versus placebo pellet) and type of injection on test (naloxone versus no injection). Injection of naloxone precipitated a somatic withdrawal syndrome among morphine pretreated rats. In situ hybridization histochemistry revealed a potent down-regulation of hippocampal GR mRNA 4 h after injection of naloxone. Levels of GR mRNA in the amygdala and hypothalamus were unchanged. Hippocampal MR mRNA levels from these same animals were unchanged. By contrast, neither chronic morphine exposure nor injection of naloxone in morphine naive animals affected GR or MR mRNA levels. These results show that during opiate dependence the levels of hippocampal GR mRNA are more sensitive to episodes of withdrawal than to chronic drug exposure and are consistent with an increased vulnerability to stress during opiate dependence.

Stress has a profound influence on opiate dependence. Animal models have revealed that stressors facilitate the: (1) behavioral impact of opiates [3,4]; (2) initiation of opiate self-administration [16,17]; (3) aversive motivational effects of opiate withdrawal [20]; and (4) relapse to opiate-seeking [16,18]. Importantly, there is evidence that this interaction between stress and drug-dependence is bidirectional. Thus, just as a history of exposure to stressors increases vulnerability to drug dependence so too can a history of drug-dependence increase vulnerability to stress [6,7,9]. Rats rendered dependent upon morphine via daily injections show contrasting patterns of enhanced hypothalamic–pituitary–adrenal (HPA) axis activation in the short term (hours to days) versus reduced HPA axis activation in the long term (days to weeks) following opiate dependence [6,7]. The enhanced activation in the short term following opiate dependence is characterised by a potentiated and prolonged secretion of corticosterone in response to restraint stress coupled with significantly reduced sensitivity to steroid negative feedback [6,7]. This enhanced response was associated with a reduction in whole brain glucocorticoid (GR) protein expression [7].

Three questions remain to be answered regarding these short-term alterations in HPA axis function. First, although this pattern of function is consistent with a loss of receptor-mediated negative feedback over the HPA axis it is unknown whether the reduction in whole brain GR protein expression reported elsewhere [7] was accompanied by a similar selective down-regulation of GR levels in the hippocampus, a key site for glucocorticoid negative feedback [5], or whether this down-regulation was nonselective across several brain regions. Second, although the daily injection regime employed in previous studies [1,6,7] mimics patterns of opiate self-administration, it does not distinguish between the influence of prolonged opiate exposure versus repeated episodes of opiate withdrawal. It is unknown whether prolonged opiate exposure or opiate withdrawal is critical for alterations in the HPA axis. Finally, it is unknown whether alterations in GR expression are accompanied by...
alterations in mineralocorticoid (MR) expression. Differences in affinity for corticosterone, differential effects of antagonists on responses to stressors, and potentially opposing influences on hippocampal neuronal function suggest that coordinated regulation of hippocampal GR and MR is critical for normal hippocampal and HPA axis function [2,5,8,10,11,13,19].

The present experiments used in situ hybridization to address these questions. Subjects were 23 naive male Sprague–Dawley rats (Charles River, Portage, MI) weighing between 250 and 300 g at the start of the experiment. Rats were housed in plastic cages, in groups of two to three, in a colony room maintained on a 12:12 h light/dark cycle (lights on at 7:00 A.M.). Food and water were freely available for the duration of the experiment. All animals were killed between 10:00 A.M. and 11:00 A.M. The procedures were in accordance with the National Institution of Health guide for the care and use of Laboratory Animals (NIH Publications No. 80-23, revised 1978) and were approved by the University of Michigan Committee on Use and Care of Animals (UCUCA).

There were four groups: Group Morphine Alone (n = 6), Group Placebo Alone (n = 6), Group Morphine–Naloxone (n = 6), and Group Placebo–Naloxone (n = 5). On Day 1, rats were implanted with two 75-mg morphine base or placebo pellets (NIDA, Bethesda, MD) under brief gas anesthesia. Five days later, rats were briefly removed from their home cages, injected s.c. with 3 mg/kg naloxone (Sigma, St. Louis, MO) or handled, and returned to their home cages. Rats were briefly inspected (5 min) to verify the presence of a withdrawal syndrome. Four hours later their brains were removed. This time point was chosen to correspond to that used by Houyshar and Woods [6] in their study of whole brain GR protein levels. Sections (10 μM) were collected using a cryostat and thaw mounted on poly-L-lysine coated slides. Tissue was air dried and stored at −80 °C until processing for in situ hybridization histochemistry. These brains are from the same animals used previously to study alterations in CRH mRNA during opiate withdrawal [12]. The rat GR probe was a 451 nucleotide fragment generated against rat GR mRNA coding region (nucleotides 2364-2815). The MR probe was a 400 nucleotide fragment generated against 3′ untranslated region of MR mRNA. The procedure for in situ hybridization has been described in detail previously [12].

Brain section images were captured digitally and the relative optical density of the autoradiographic film was determined using NIH Image software. Measurements were taken from a series of 8–12 sections through the rostral–caudal extent of the structures of interest, except the hypothalamus where measurements were based on a series of 5–7 sections. The optical density values were corrected for background (by defining signal as that activation which exceeded 3.5 standard deviation units above the mean gray value of background), multiplied by the total area of the signal (number of pixels) above background in the sample, and averaged across sections to produce one data point for each brain region per animal.

Injection of naloxone in morphine-pelleted rats produced signs of somatic withdrawal in all rats (e.g., wet-dog shakes, ptosis, diarrhoea). These were not quantified because previous work from our laboratory has described this syndrome in full [12] and it was important to leave the rats undisturbed in their home cages until brains were removed. Fig. 1 shows the

![Fig. 1](image_url)

**Table 1**

Mean and S.E.M. (in parentheses) optical density measurements of GR mRNA

<table>
<thead>
<tr>
<th>Group</th>
<th>Dentate gyrus</th>
<th>CA1</th>
<th>Hypothalamus</th>
<th>Amygdala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo alone</td>
<td>21,650</td>
<td>19,181</td>
<td>2832</td>
<td>6113</td>
</tr>
<tr>
<td></td>
<td>(2856)</td>
<td>(2294)</td>
<td>(370)</td>
<td>(852)</td>
</tr>
<tr>
<td>Morphine alone</td>
<td>28,621</td>
<td>22,256</td>
<td>4199</td>
<td>6288</td>
</tr>
<tr>
<td></td>
<td>(2587)</td>
<td>(1935)</td>
<td>(275)</td>
<td>(794)</td>
</tr>
<tr>
<td>Placebo–naloxone</td>
<td>26,829</td>
<td>16,815</td>
<td>7316</td>
<td>5502</td>
</tr>
<tr>
<td></td>
<td>(1576)</td>
<td>(3104)</td>
<td>(322)</td>
<td>(336)</td>
</tr>
<tr>
<td>Morphine–Naloxone</td>
<td>14,212*</td>
<td>7022*</td>
<td>3968</td>
<td>4275</td>
</tr>
<tr>
<td></td>
<td>(896)</td>
<td>(850)</td>
<td>(702)</td>
<td>(502)</td>
</tr>
</tbody>
</table>

*Indicates significant difference (p<0.05) from the remaining three groups.
distribution of GR (Panel A) and MR (Panel B) mRNA in the hippocampus from representative sections. Table 1 shows the mean and standard error of the mean (S.E.M.) optical density measurements of GR mRNA and Table 2 shows mean and S.E.M. optical density measurements of MR mRNA.

The $2 \times 2$ ANOVA of optical density measurements for total hippocampal GR mRNA failed to reveal a significant main effect of type of pellet pretreatment (morphine versus placebo) ($p>0.05$), but did reveal a significant main effect for type of injection on test (naloxone versus no injection) ($p=0.003$). Moreover, the $2 \times 2$ interaction was significant ($p<0.0001$), indicating that the effect of naloxone injection on GR mRNA was selective to rats pretreated with morphine pellets. This interpretation was confirmed by post-hoc comparisons: GR mRNA was significantly lower among Group Morphine–Naloxone as compared to each of the remaining three groups ($p<0.05$), but these three groups did not differ from each other ($p>0.05$). These findings are unaltered if GR mRNA in the dentate gyrus and CA1 field are considered separately. GR mRNA was also detected in the thalamus and hypothalamus as well as medial and central nuclei of the amygdala. The $2 \times 2$ ANOVAs failed to detect any significant effects for type of pellet pretreatment (morphine versus saline), type of injection on test (naloxone versus no injection), or any $2 \times 2$ interactions ($p>0.05$) in these structures.

The $2 \times 2$ ANOVA of optical densities of total hippocampal MR mRNA levels failed to reveal a significant main effect of type of pellet pretreatment (morphine versus placebo) ($p>0.05$), failed to reveal a significant main effect for type of injection on test (naloxone versus no injection) ($p>0.05$), and failed to reveal a significant two-way interaction ($p>0.05$). These findings are unaltered if MR mRNA in the dentate gyrus, CA1, CA2, and CA3 fields are considered separately.

This experiment addressed three questions regarding HPA axis function during opiate dependence. The first was whether opiate dependence was associated with a selective down-regulation of GR gene expression in the hippocampus. The results showed that precipitated withdrawal from morphine was associated with a potent down-regulation of hippocampal GR mRNA that was not observed in other brain regions studied (amygdala, thalamus, hypothalamus). The second question was whether this reduction in hippocampal GR mRNA was a consequence of prolonged opiate exposure or whether it depended upon episodes of opiate withdrawal. This is important because the daily morphine injection regime employed in previous studies [1,6,7] does not distinguish between the effects of chronic opiates versus those of the withdrawal subsequent to these injections. The results showed that levels of hippocampal GR mRNA are more sensitive to episodes of opiate withdrawal than to chronic opiate exposure. This finding is consistent with the effects of these manipulations on plasma glucocorticoid levels. Under present conditions, rats implanted with morphine pellets (chronically treated with morphine but in the absence of explicit episodes of withdrawal) do not differ in basal plasma corticosterone levels from control animals whereas precipitated withdrawal from morphine is associated with a potent and prolonged increase in plasma glucocorticoid levels [12]. Given that increases in plasma corticosterone decrease levels of hippocampal GR mRNA [14,15], it is tempting to speculate that increased corticosterone during opiate withdrawal [12] is critical for down-regulation of hippocampal GR. Further experimentation is needed to address this possibility and to determine the time course of the changes observed here. The third question was whether alterations in GR mRNA were accompanied by alterations in MR mRNA. There was no evidence here for such alterations. These results, when combined with previous demonstrations of an up-regulation of CRH mRNA in the PVN from the same brains [12], identify precise mechanisms for alterations in HPA axis function observed during opiate withdrawal [6,7].

Acknowledgements

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References


