Alteration in Regulation of Serotonin Release in Rat Dorsal Raphe Nucleus after Prolonged Exposure to Morphine

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ABSTRACT

Regulation of serotonin (5-HT) release may be altered during the development of opioid tolerance and dependency. To test this hypothesis, changes in extracellular 5-HT during prolonged administration of morphine were determined by microdialysis in the dorsal raphe nucleus (DRN) of freely behaving rats. Morphine or placebo pellets were implanted s.c. As compared to placebo, morphine pellets induced a sustained, ~50% increase in DRN 5-HT and a significant elevation in hot plate latency during the 12-hr period after implantation. One week later DRN 5-HT had returned to control levels, and implanting additional morphine pellets had no effect on 5-HT or hot plate latency.

Some evidence suggests that a change in serotonergic neurotransmission is involved in opioid tolerance and dependency. Acute systemic administration of morphine enhanced 5-HT turnover in the mammalian CNS, but the increase in turnover was attenuated after prolonged opiate treatment and tissue levels of 5-HT were decreased during withdrawal (Yarbrough et al., 1973). Opioids do not directly stimulate serotonergic neuronal discharge (Auerbach et al., 1985; Chiang and Pan, 1985; Jolas and Aghajanian, 1997). Instead, similar to the indirect excitatory effect on dopaminergic cells (Johnson and North, 1992) opioids may disinhibit serotonergic neuronal activity. In support of this hypothesis, opioids suppressed GABA-mediated inhibitory postsynaptic currents recorded in vitro from serotonergic neurons in the rat DRN (Jolas and Aghajanian, 1997). Although, excitatory postsynaptic currents were similarly suppressed by opioids in vitro (Jolas and Aghajanian, 1997), GABA appears to be the predominant tonic influence on serotonergic neurons in the rat DRN in vivo (Tao and Auerbach, 1994; Tao et al., 1997). Thus, the net short term effect of morphine may be an increase in 5-HT release in widespread areas of the forebrain (Tao and Auerbach, 1994, 1995).

One day after removing the pellets from rats exposed to morphine for 2 wk, acute challenge with morphine (20 mg/kg, s.c.) had a significantly smaller effect on 5-HT in the DRN as compared to the placebo treatment group. Administration of naloxone to rats implanted with morphine pellets for 2 wk induced signs of withdrawal and a significant decrease in DRN 5-HT. These results suggest that the regulation of 5-HT release is altered during the development of tolerance to morphine. Thus, DRN 5-HT may be one of the factors involved in the changes in physiology and behavioral state during opioid withdrawal.

After prolonged administration of opioids, inhibitory influences on monoaminergic neurons may be up-regulated. This hypothesis is suggested by the increase in GABA-mediated inhibition of dopaminergic neurons in the ventral tegmental nucleus during withdrawal from morphine (Bonci and Williams, 1997). Similarly, after prolonged treatment with morphine, single neurons recorded in the ventrolateral PAG showed signs of adaptation (Chiang and Christie, 1996). Thus, there was a reduction in the direct inhibitory effect of opioids on PAG neurons in slices prepared from morphine-dependent rats. Conversely, naloxone-precipitated withdrawal was associated with increased neuronal discharge. There is no conclusive evidence that this subpopulation of opioid-sensitive PAG neurons is GABAergic or makes synaptic contact with serotonergic neurons. Nevertheless, these results are consistent with the possibility that GABA release in the adjacent DRN increases during withdrawal from morphine, which in turn could result in decreased serotonergic neuronal activity.

Because 5-HT may be involved in adaptation to stress and drug addiction, it is of interest to determine if prolonged exposure to opioids alters serotonergic neuronal activity. However, earlier turnover and electrophysiological studies have provided conflicting evidence concerning this issue (reviewed by Redmond and Krystal, 1984). To test the specific

ABBREVIATIONS: 5-HT, serotonin (5-hydroxytryptamine); 5-HIAA, 5-hydroxyindoleacetic acid; GABA, γ-aminobutyric acid; DRN, dorsal raphe nucleus; MRN, median raphe nucleus; aCSF, artificial cerebrospinal fluid; MPE, maximum possible effect; PAG, periaqueductal gray; CNS, central nervous system.
hypothesis that opioid tolerance and dependency is associated with alterations in regulation of 5-HT release, we have carried out microdialysis measurements in the DRN of unanesthetized rats. The DRN is of particular interest because forebrain sites selectively innervated by this group of serotonergic cell bodies are most sensitive to the disinhibitory effects of morphine and GABA_A receptor antagonists (Tao and Auerbach, 1995; Tao et al., 1996). Opioid tolerance was produced by s.c. implantation of morphine pellets. Changes in 5-HT were measured immediately after implanting the pellets, and after 1 and 2 wk of continuous exposure to morphine. Changes in 5-HT were also determined during naltrexone-precipitated withdrawal in morphine-dependent animals. Consistent with our hypothesis, extracellular 5-HT was first increased and then decreased back to baseline during continuous administration of morphine for 2 wk. Furthermore, during naltrexone-precipitated withdrawal, extracellular 5-HT decreased below control levels.

Methods

Animal preparation. Male Sprague Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, IN) were individually housed in cages with food and water available ad libitum. All animal use procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Rutgers University Institutional Review Board. The animals were kept at least two weeks on a reversed light-dark cycle (lights off from 9:30 A.M. to 9:30 P.M.) and were briefly handled three times a week. Rats were kept in cages with food and water available ad libitum. All animal use procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Rutgers University Institutional Review Board. The animals were kept at least two weeks on a reversed light-dark cycle (lights off from 9:30 A.M. to 9:30 P.M.) and were briefly handled three times a week. Rats weighing 300 to 350 g were anesthetized with a combination of xylazine (4 mg/kg, i.p.) and ketamine (80 mg/kg, i.p.), and then mounted in a Kopf stereotaxic frame in the flat skull position. Guide cannulas, 10 mm in length (22-gauge stainless steel tubing), were implanted at a 32° angle lateral to midline. The coordinates for the tip of the DRN guide cannulas were: AP 1.2 and ML 4.0 relative to interaural zero and DV .9 mm below the skull surface (Paxinos and Watson, 1986).

Microdialysis and analytical techniques. Concentric style (I-shaped) microdialysis probes were constructed from 26-gauge stainless steel tubing and glass silica. The dialysis tubing was hollow nitrocellulose fiber (0.2 mm o.d., 6000 MW cut-off; Spectrum Medical Industries, Los Angeles, CA). The length of the steel shaft was adjusted to place 1.0-mm long segments of dialysis tubing in the DRN (DV 5.5–6.4, 32° angle).

Experiments were begun 1 wk or more after surgery. The night before an experiment, rats were briefly anesthetized with methoxyflurane, and dialysis probes were inserted through the guide cannulas and secured with dental cement. Animals were attached to a fluid swivel, allowing unrestricted behavior within the testing chamber. Before collecting samples, dialysis probes were perfused overnight with aCSF containing 140 mM NaCl, 3.0 mM KCl, 1.5 mM CaCl_2, 1.0 mM MgCl_2, 27 mM NaH_2PO_4, 1.2 mM Na_2HPO_4, 1.0 μM citalopram, pH 7.4. The aCSF was pumped at a rate of 1.0 μl/min. Sample collection began at the start of the lights-off period under dim red light conditions. The rationale for collecting samples during the lights-off period is that rats are a nocturnally active species and serotonergic neuronal discharge is presumably higher during waking behavior than during sleep (Jacobs and Fornal, 1995).

Samples were collected every 30 min and analyzed within 30 min of collection by high-performance liquid chromatography with electrochemical detection, as previously described in detail (Auerbach et al., 1989). Separation of 5-HT from other electroactive compounds was achieved on a 10 cm × 3.2 mm column with ODS 5 μm packing (BAS Inc., W. Lafayette, IN). Mobile phase (composition 0.15 M chloroacetic acid, 0.12 M sodium hydroxide, 0.18 mM EDTA, 56 mM/liter of acetonitrile and 1.0 mM sodium octane sulfonic acid was pumped at a rate of 0.9 ml/min. Monoamines were measured using a dual potentiostat electrochemical detector (EG&G PARC, Princeton, NJ) and dual glassy carbon working electrodes in the parallel configuration. Applied potentials, relative to an Ag/AgCl reference electrode, were set at approximately maximal and half-maximal for oxidation of 5-HT. These values were checked frequently and were usually about 590 and 530 mV. The detection limit for 5-HT was approximately 300 fg, based on a signal-to-noise ratio of 3:1.

Experimental protocol. Drugs were administered to rats after 5-HT levels in four successive samples were stable (less than ±10% fluctuation of baseline). Morphine pellets (each containing 75 mg free base) or placebo pellets were implanted s.c. in rats briefly anesthetized with methoxyflurane. Tests were carried out at four different times: 1) Rats were tested before and immediately after implanting pellets on day 1; 2) rats implanted with pellets on day 1 were tested immediately before and after implanting additional pellets on day 7; 3) pellets were implanted on days 1 and 7, and were removed on day 14. The effect of a challenge dose of morphine (20 mg/kg, s.c.) was tested 24 hr later and 4) rats were implanted with pellets on days 1 and 7. With the pellets still implanted, rats were tested on day 15 before and after naltrexone-precipitated withdrawal.

Quantification of analgesia. Rats were placed on a hot plate analgesiometer (Omnitech, Columbus, OH) set to 55°C. Latencies to thermal stimulation were determined by the time of paw-licking or hopping, with a cut-off of 45 sec. The data were expressed as mean

Fig. 1. Coronal section at AP 1.2 mm relative to the interaural line based on the rat brain atlas of Paxinos and Watson (1986). A, Photomicrograph showing a probe track, indicated by the arrow, within the DRN. B, Tracing of a coronal section, AP 1.2 mm relative to the interaural line, illustrating the location of the dialysis membrane tracks for the experiment shown in figure 2B. The bars represent the area stained by diffusion of the fast green dye from the dialysis probe membrane. Abbreviations: PAG, periaqueductal gray; DRN, dorsal raphe nucleus; SCF, superior cerebellar peduncle.
Table 1
Extracellular 5-HT levels in the DRN; data are expressed as pg (mean ± S.E.M.) in 30 μl of dialysate; n = 5–8 (except where noted)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Baseline before pellet implantation</th>
<th>Placebo</th>
<th>Morphine</th>
</tr>
</thead>
<tbody>
<tr>
<td>All rats</td>
<td></td>
<td>5.7 ± 0.7 (n = 24)</td>
<td></td>
</tr>
<tr>
<td>After pellet implantation</td>
<td>2 pellets</td>
<td>5.0 ± 1.3</td>
<td>8.7 ± 1.3^a</td>
</tr>
<tr>
<td></td>
<td>4 pellets</td>
<td>6.5 ± 0.8</td>
<td>12.6 ± 2.5^a</td>
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Experiment 2
Baseline on day 7 (pellet residue still implanted)
| 2 pellets | 5.0 ± 1.5 | 4.5 ± 1.0 |
| 4 pellets | 6.4 ± 0.8 | 5.8 ± 0.9 |

After additional pellets on day 7
| 2+2 pellets | 7.9 ± 1.4 | 5.8 ± 1.3 |
| 4+4 pellets | 5.1 ± 0.9 | 4.9 ± 0.8 |

Experiment 3
Baseline on day 15 (24 h after removal of pellet residues)
| 4+4 pellets | 5.6 ± 1.6 | 6.0 ± 2.2 |
| After acute challenge with morphine (20 mg/kg, s.c.) on day 15
| 4+4 pellets | 9.4 ± 3.1b | 6.7 ± 2.4 |

Data were analyzed with one-way ANOVA followed by Scheffe’s F test.
^a P < .05, effect of morphine pellets vs. placebo pellets.
^b P < .05, comparison of the effect of systemic morphine on rats implanted with placebo pellets vs. morphine pellets.

Results

Experiment 1: Increased 5-HT and analgesia on the first day of morphine pellet implantation. After collecting baseline samples, rats were briefly anesthetized with methoxyflurane and implanted with two or four morphine or placebo pellets. DRN 5-HT was significantly increased between 1 to 1.5 hr after implanting morphine pellets and was still elevated in the last sample collected, 12 hr after morphine administration (fig. 2A). For both two and four mor-

Fig. 2. Time-course of elevated extracellular 5-HT in the DRN after s.c. implantation of morphine pellets. As described in methods, the data (mean ± S.E.M.) were expressed as a percentage of the average baseline level. Baseline was calculated as the average of the last four baseline samples before drug administration. A, The arrow indicates the time of morphine (75 mg each) or placebo pellet implantation. DRN 5-HT was significantly increased for at least 12 hr compared to placebo controls (two pellets, F(1, 8) = 31.4, P < .001; four pellets, F(1, 12) = 33.6, P < .001). B, The arrow indicates the time when four morphine pellets (75 mg each) were implanted. The horizontal bar indicates the period when the GABA<sub>A</sub> receptor agonist muscimol was infused into the DRN. The morphine-induced increase in 5-HT was significantly reduced during infusion of muscimol as compared with the pre-morphine baseline [F(1, 6) = 17.9, P < .01], or with 5-HT after implantation of the morphine pellets [F(1, 6) = 46.4, P < .001].

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Materials. All chemicals were reagent grade or better. Morphine base pellets, placebo pellets and morphine sulfate powder were provided by the National Institute on Drug Abuse. Citalopram hydrobromide was provided courtesy of Dr. C. Sanchez (H. Lundbeck A/S, Copenhagen-Valby). Naltrexone hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO), and muscimol hydrobromide from RBI (Natick, MA).
phine pellets, the mean increase in 5-HT was about 50% above levels in the placebo treatment groups. Absolute levels of 5-HT in baseline samples and after pellet implantation are shown in table 1.

Reverse dialysis infusion of the GABA_4 receptor agonist muscimol into the DRN was used to determine if extracellular 5-HT was dependent on depolarization-induced release. As shown in figure 2B, 2 hr after implanting four morphine pellets, extracellular 5-HT was increased about 50% above baseline. At this time, during infusion of muscimol (100 μM) for 1 hr, extracellular 5-HT was reduced to about 40% of the original, pre-morphine baseline. During the period of muscimol washout, extracellular 5-HT returned to a level above the pre-morphine baseline level.

To quantitate the analgesic response, a separate group of rats was briefly anesthetized with methoxyflurane for implantation of pellets. Using the hot plate test, the analgesic response was tested once every hour between 1 to 12 hr. As shown in figure 3, latencies were elevated between 1 to 12 hr. In response to two morphine pellets, mean hot plate latency was 23.1 ± 3.4 sec as compared to 11.3 ± 0.7 sec for rats implanted with two placebo pellets (fig. 2A). This increase in response to morphine, 34.9% of the MPE, was significant in comparison to the placebo group (P < .01, Mann-Whitney U test). In response to four morphine pellets, mean latency was increased to 41.1 ± .7 sec as compared to 8.5 ± 0.5 sec for rats implanted with four placebo pellets (fig. 2B). This pronounced increase in response to four morphine pellets, 92.7% MPE, was significant in comparison to the placebo group (P < .01, Mann-Whitney U test).

Experiment 2: Tolerance to the effect of morphine pellets 1 wk after implantation. On the evening of the sixth day, with the original pellets in place, rats were implanted with dialysis probes in the DRN. Samples were taken the next morning, and as shown in table 1, there were no significant differences in baseline 5-HT between the placebo and morphine treatment groups. After obtaining baseline samples, rats were briefly anesthetized and implanted with two or four more pellets. After one week exposure, rats were tolerant to the effect of implanting additional morphine pellets. There was no significant increase in DRN 5-HT in response to either two or four additional morphine pellets (table 1; fig. 4). Similarly, additional morphine pellets had no effect on hot plate latencies of the chronic morphine treatment groups (fig. 5). The changes in MPE were -3% (P > .05, Mann-Whitney U test) and 5% (P > .05, Mann-Whitney U test) after implantation of two or four additional morphine pellets, respectively.

Experiment 3: Attenuated effect of morphine injection, 1 day after removal of morphine pellets. Four pellets were implanted on day 1, and four more on day 7 as described in “Methods.” Previous research indicates that ~8 hr is sufficient for complete elimination of morphine from plasma and brain after removing chronically implanted pellets (Bhargava, 1978). Thus, pellet residues were removed on day 14, and baseline samples were taken the next day. As shown in table 1, there were no significant differences in baseline 5-HT between the morphine and placebo treatment groups. After determining baseline levels, rats received injections with morphine (20 mg/kg, s.c.) or the saline vehicle. For the chronic morphine treatment group, injection of morphine in comparison to the saline vehicle had no significant effect on DRN 5-HT (fig. 6). In contrast, morphine injection produced a significant increase in 5-HT in the DRN of the placebo pellet group (fig 6).
Experiment 4: Naltrexone-precipitated withdrawal results in decreased 5-HT. Rats were implanted with four pellets on day 1, and four more pellets on day 7 as described in “Methods.” On day 15 with the pellet residues in place, all rats received injections with naltrexone (20 mg/kg, s.c.). Naltrexone injection-induced withdrawal behaviors such as diarrhea and tremor in rats implanted with morphine pellets (data not quantified). As shown in figure 7, naltrexone elicited about a 30% reduction in DRN 5-HT \[F(1, 10) = 8.985, P = .0134\]. The maximal decrease was 2.5 ± .5 pg/sample from the baseline level of 7.6 ± 1.3 pg/sample. The decrease was sustained for at least 3 hr after naltrexone injection.

Discussion

The major aim of this study was to test the hypothesis that the regulation of 5-HT release is altered during the development of opioid tolerance and dependence. Consistent with this hypothesis, extracellular 5-HT in the rat DRN was increased immediately after implantation of morphine pellets, but returned to baseline levels during 1 wk of exposure. After prolonged treatment, DRN 5-HT was unresponsive to additional pellets or systemic injection of morphine, and decreased during naltrexone-precipitated withdrawal. These results are in agreement with reports that the increase in cerebral 5-HT turnover produced by acute morphine challenge was attenuated after chronic treatment (Yarbrough et al., 1973; Ahtee, 1980), and whole brain tissue levels of 5-HT were decreased during withdrawal (Ahtee, 1980).

Acute effects of morphine. Increases in extracellular 5-HT were small but sustained for at least 12 hr in the DRN.
of naive rats implanted with morphine pellets. Acute systemic injection of morphine also produced a significant but more transient increase in DRN 5-HT. Presumably, these changes in DRN 5-HT provide an indication of the effect of morphine on 5-HT release in the forebrain. In support of this assumption, systemic injection of morphine produced similar increases in extracellular 5-HT in the DRN and the nucleus accumbens, a forebrain site innervated by the DRN (Tao and Auerbach, 1995). Furthermore, local infusion of morphine into the DRN resulted in increased extracellular 5-HT in the nucleus accumbens (Tao and Auerbach, 1995).

The stable elevation in hot plate latencies indicates that pellet implantation resulted in analgesic levels of morphine. Although two and four morphine pellets produced similar increases in DRN 5-HT, the higher dose had a significantly greater effect on hot plate latency. Thus, morphine had a maximal effect on DRN 5-HT at a dose lower than necessary for producing strong analgesia. This provides evidence of a dissociation between the stronger antinociceptive effect of four morphine pellets and increased activity of DRN 5-HT neurons, consistent with other studies suggesting that serotonergic neurons are not involved in opioid-induced analgesia (Auerbach et al., 1985; Gao et al., 1998). The DRN and surrounding PAG have been implicated in responses to stress and modulation of pain (reviewed by Wang and Nakai, 1994). For example, inescapable but not escapable shock enhanced the analgesic response to threshold doses of morphine and this effect was blocked by infusion of a 5-HT autoreceptor agonist, 8-OH-DPAT, into the DRN (Sutton et al., 1997).

These studies suggest that, not as a consequence of arousal, but related to a specific behavioral state produced by unavoidable stress, opioids can modulate pain sensitivity via an interaction with DRN serotonergic neurons. However, according to some reports, 5-HT release and neuronal activity in response to morphine and various stressors were not greater than levels observed during undisturbed waking behavior or presentation of appetitive stimuli (Auerbach et al., 1985; Rueter and Jacobs, 1996; Gao et al., 1998). Thus, Jacobs and Fornal (1995) suggest that 5-HT plays a more general role in modulating sensitivity to somatosensory stimuli across changes in behavioral state. For example, increased serotonin release during active waking behavior might have a tonic influence on somatosensory transmission that enhances the analgesic effect of opioids.

The preponderance of evidence suggests that opioids do not directly excite DRN serotonergic neurons. Instead, they may inhibit GABAergic afferent activity and thus elicit small increases in serotonergic neuronal activity and release (Tao and Auerbach, 1994; Stiller et al., 1996; Jolas and Aghajanian, 1997). However, opioids also suppressed excitatory postsynaptic currents in DRN serotonergic neurons (Jolas and Aghajanian, 1997). Thus, the net effect of opioids may depend on the balance between GABAergic and glutamatergic tone on serotonergic neurons. Glutamatergic inputs do not have a major tonic influence on DRN serotonergic neurons (Levine and Jacobs, 1992; Tao et al., 1996, 1997), but instead may be involved in the small transient activation elicited by phasic sensory stimuli (Levine and Jacobs, 1992). In contrast, GABA had a strong and sustained inhibitory influence on serotonergic neurons in the DRN of unanesthetized, undisturbed rats (Tao et al., 1996). This preponderance of tonic GABAergic over glutamatergic influence on serotonergic neurons in the DRN is presumably necessary for the disinhibitory influence of opioids under our experimental conditions. However, because GABA release in the DRN may decrease during wakefulness (Levine and Jacobs, 1992; Nitz and Siegel, 1997), differences in behavioral state could be a factor in the variable effects of opioids on extracellular 5-HT in other microdialysis studies (Matos et al., 1992). If GABA release is already low during behavioral arousal, opioid administration might have no effect or produce a decrease in 5-HT neuronal activity (Auerbach et al., 1985). Relatedly, anesthetics can interfere with the effect of opioids on 5-HT (Chiang and Xiang, 1987; Rivot et al., 1988), presumably by disrupting the influence of GABAergic neurotransmission in the raphe (Tao and Auerbach, 1994).

It is important to note that direct infusion of morphine into the MRN had no apparent effect on 5-HT in the MRN or forebrain sites selectively innervated by MRN serotonergic neurons (Tao and Auerbach, 1995). Similarly, systemic administration of morphine did not stimulate serotonergic neurons in the nucleus raphe magnus (Auerbach et al., 1985; Gao et al., 1998). Furthermore, a subpopulation of DRN serotonergic neurons was directly inhibited by opioids (Jolas and Aghajanian, 1997). Also, infusion of opioids into the hippocampus reduced extracellular 5-HT in this forebrain site (Yoshioka et al., 1993). Thus, although we have observed increases in extracellular 5-HT in the DRN and several forebrain areas innervated by the DRN (Tao and Auerbach, 1995), in other sites, there may be no change or a decrease in 5-HT in response to opioids.

It is possible that increases in DRN 5-HT in response to morphine were in part the result of state-related changes in serotonergic neuronal activity, and not due to a direct effect of opioids in the ventral PAG. The time spent in slow wave and desynchronized sleep is reduced by opioids (Kay et al., 1979), and during waking behavior the activity of serotonergic neurons increases (Jacobs and Fornal, 1995). Thus, increased 5-HT in microdialysis samples may be just a consequence of opioid-induced attenuation of sleep. However, contrary to this hypothesis, local infusion of morphine into the MRN produced strong behavioral arousal and increased locomotor activity, but this was not associated with an increase in extracellular 5-HT in either the medial septum, an MRN projection site, or a DRN projection site, the nucleus accumbens (Tao and Auerbach, 1995). Furthermore, infusion of morphine into the DRN also produced behavioral arousal and increased extracellular 5-HT in the nucleus accumbens, but had no effect on 5-HT in the medial septum. This sitespecific pattern of increased 5-HT (see also Snelgar and Vogt, 1980; Spampinato et al., 1985) and lack of correlation to change in arousal or locomotor activity indicates these changes are not secondary to an effect of opioids on behavioral state. Instead these results suggest that altered 5-HT release is one cause of the behavioral state elicited by morphine.

Methodological considerations. Our assumption that extracellular 5-HT in the raphe provides an indication of serotonergic neuronal activity and release in the forebrain is based in part on previous evidence. Raphe 5-HT was decreased in response to either systemic administration or local infusion of 8-OH-DPAT, a somatodendritic 5-HT autoreceptor agonist that strongly inhibits serotonergic neuronal activity (Bosker et al., 1994; Tao and Auerbach, 1996). Simi-
larly, local infusion of TTX greatly reduced 5-HT in the raphe (Bosker et al., 1994; Tao et al., 1997). Furthermore, TTX infusion into the DRN produced a decrease in extracellular 5-HT in the nucleus accumbens (Tao et al., 1997). By blocking voltage-dependent sodium channels, TTX inhibits the propagation of action potentials. In our study, we infused the GABA<sub>A</sub> receptor agonist muscimol into the DRN after morphine pellet implantation. Activation of GABA<sub>A</sub> receptors in the DRN inhibits serotonergic neuronal discharge (Innis and Aghajanian, 1987), and 5-HT release in the DRN and forebrain sites (Tao et al., 1996). The observed decrease in 5-HT below pre-morphine levels suggests that baseline extracellular 5-HT and the opiate-induced increase were dependent on 5-HT neuronal activity. Together, these results provide evidence that extracellular 5-HT in the DRN is mainly derived from depolarization-induced release and is a reflection of 5-HT release in forebrain sites. One exception to this general conclusion is the effect of infusing 5-HT reuptake blockers into the raphe. This produces increased extracellular 5-HT in the raphe but decreased 5-HT in the forebrain as a consequence of autoreceptor-mediated inhibition serotonergic neuronal activity (Romero and Artigas, 1997). In contrast, morphine in the DRN presumably induced an increase in serotonergic neuronal activity and thus, increased release from terminals in forebrain projection sites and in the DRN. In summary, it appears that extracellular 5-HT in the DRN is dependent on depolarization and generally changes in parallel with increases and decreases in serotonergic neuronal activity.

It is also important to note that the dorsal-ventral and medial-lateral dimensions of the DRN are relatively small. Thus, it is possible that some of the sampled 5-HT may have been released in adjacent structures such as the ventrolateral PAG and other nuclei in the pontine tegmentum. However, if histological examination indicated that a probe site was completely outside of the DRN, data were eliminated from consideration. Furthermore, because of the steep decrease in concentration at short distances from the point of release, microdialysis mainly samples neurotransmitter released close to the probe membrane surface (Bungay et al., 1990).

**Tolerance to the effects of morphine.** Tolerance developed during continuous exposure to morphine. In addition to the loss of the analgesic response, 5-HT in the DRN had returned to control levels 1 wk after implanting morphine pellets. Implanting additional morphine pellets had no influence on analgesia or 5-HT. Also, after 2 wk of exposure, the elevation in DRN 5-HT produced by systemic injection of morphine was greatly attenuated. These results are consistent with some reports that the acute effect of morphine on 5-HT turnover is attenuated after chronic treatment (Yarbrough et al., 1973; Ahtee, 1980).

Previous research indicates that the attenuated effect of morphine during prolonged treatment is not due to a change in opiate pharmacokinetics. After implantation of two standard morphine pellets, plasma levels are elevated for up to 12 days at concentrations between 100 to 200 ng/ml (Yoburn et al., 1985; Gold et al., 1994). This is above the level that produced analgesia as determined by rat tail flick latencies (Matos et al., 1995). Tolerance to opioid analgesia begins between 12 and 24 hr after implanting morphine pellets (Gold et al., 1994). We did not measure extracellular 5-HT or analgesic response in the period between 12 hr and 1 wk after pellet implantation. Thus, further experiments are necessary to determine if tolerance to the effect of morphine on 5-HT and pain develops at the same time.

**Opioid dependency.** The behavioral syndrome associated with opioid withdrawal in rats has been thoroughly characterized. Symptoms of withdrawal can be precipitated by opioid receptor antagonists as early as 3 hr after implanting two morphine pellets and intensify with longer exposure (Gold et al., 1994). Although we did not quantitate specific behavioral or physiological symptoms, a severe withdrawal syndrome was evident when naltrexone was administered to rats implanted with morphine pellets for 15 days.

The PAG is a brain site implicated in opioid withdrawal (Bozarth and Wise, 1984; Nestler and Aghajanian, 1997). An increase in GABA release in the ventral tegmental area is involved in reduced activity of dopamine neurons during withdrawal from morphine (Bonci and Williams, 1997). Also, intrinsic adaptations in dopamine neurons may play a role in reduced dopamine release in the nucleus accumbens and thus, drug craving (reviewed by Nestler and Aghajanian, 1997). Increased activity of locus ceruleus noradrenergic neurons contributes to other behavioral and physiological symptoms of withdrawal (Rasmussen, et al., 1990). The possibility that decreased serotonergic neurotransmission is also involved in opioid dependency is supported by our results, and evidence that direct and indirect 5-HT receptor agonists can partially attenuate symptoms of withdrawal (Romandini et al., 1984).

In summary, our results provide evidence that regulation of 5-HT release in the forebrain is altered during prolonged exposure to morphine. Further studies are necessary to determine if increased activity of inhibitory afferents, or other mechanisms, such as a change in intrinsic properties of serotonergic neurons, is involved in the development of opioid tolerance and dependency. Via its role in modulating neuronal excitability in motor nuclei, thalamus, cortex and many other CNS sites, alterations in serotonergic neurotransmission could contribute to the changes in physiology, sensorimotor function and behavioral state elicited by opioids.

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**References**


