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The GABA_B receptor agonist baclofen administered into the median and dorsal raphe nuclei is rewarding as shown by intracranial self-administration and conditioned place preference in rats

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Abstract

Rationale—The midbrain raphe regions have long been implicated in affective processes and disorders. There is increasing evidence to suggest that the median (MR) and dorsal raphe nuclei (DR) tonically inhibit reward-related processes.

Objectives—Stimulation of GABA_B receptors in the midbrain raphe nuclei is known to inhibit local neurons, especially serotonergic neurons. We sought to determine if injections of the GABA_B receptor agonist baclofen into the MR or DR are rewarding, using intracranial self-administration and conditioned place preference.

Results—Rats quickly learned to lever press for infusions of baclofen (0.1–2.5 mM) into the MR, but not the ventral tegmental area or central linear nucleus. Rats increased lever pressing associated with intra-DR baclofen infusions, but not readily. Baclofen self-administration into the MR or DR was attenuated by coadministration of the GABA_B receptor antagonist SCH 50911 (1 mM) or systemic pretreatment with the dopamine receptor antagonist SCH 23390 (0.025 mg/kg, i.p.). In addition, intra-DR and intra-MR injections of baclofen induced conditioned place preference; injection into DR was more effective.

Conclusions—Baclofen injections into the midbrain raphe nuclei are rewarding. Baclofen was more readily self-administered into the MR than into the DR, while baclofen injections into the DR more readily induced conditioned place preference than those into the MR. These sites may be differentially involved in aspects of reward. These findings suggest that MR or DR neurons containing GABA_B receptors are involved in tonic inhibitory control over reward processes.

Keywords

Intracranial self-administration; Conditioned place preference; Reward; Reinforcement; Baclofen; GABA_B receptors; Median and dorsal raphe nuclei

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Introduction

The dorsal (DR) and median raphe nuclei (MR) have long been implicated in affective processes and psychiatric conditions such as mood and anxiety disorders (Cools et al. 2008; Maier and Watkins 2005). DR and MR contain serotonergic neurons that provide primary source of forebrain serotonin. Accumulating evidence suggests that MR and DR neurons are involved in inhibitory control over reward processes. Using the serotonin 5-HT_{1A} receptor agonist 8-OH-DPAT, which inhibits serotonergic neurons by stimulating autoreceptors, Fletcher et al. (1993) found that pairing a specific environment with 8-OH-DPAT injections into the MR or DR leads to subsequent preference for that location, called “conditioned place preference”. Moreover, 8-OH-DPAT injections into the MR facilitate rewarding effects of electrical stimulation at the lateral hypothalamic area (Fletcher et al. 1995). More recently, we found that inhibiting MR or DR neurons by locally administering the GABA_A receptor agonist muscimol is rewarding (Liu and Ikemoto 2007): Rats learn to self-administer muscimol into the MR or DR; in addition, muscimol injections into the MR induce conditioned place preference.

The rewarding effects of muscimol injected into the midbrain raphe nuclei may be mediated by the dopaminergic system. Muscimol injections into the median raphe nucleus increase the ratios of 3,4-dihydroxyphenylacetic acid (DOPAC) or homovanillic acid (HVA) to dopamine in postmortem accumbens tissues (Wirtshafter and Trifunovic 1992), suggesting that the manipulations increase extracellular dopamine levels in the nucleus accumbens. Consistently, self-administration of muscimol into the MR is readily disrupted by a low dose of systemic administration of dopamine receptor antagonists (Liu and Ikemoto 2007).

The MR and DR also contain GABA_B receptors (Abellán et al. 2000a; Smith and Gallager 1987; Wirtshafter and Sheppard 2001), which may be involved in reward processes as GABA_A receptors. Injections of the GABA_B receptor agonist baclofen into the MR facilitate open-field locomotor activity, feeding, and drinking, effects that are also elicited by injections of muscimol into the region (Kliteneck and Wirtshafter 1988; Przewlocka et al. 1979; Wirtshafter et al. 1993). Therefore, we hypothesized that injections of baclofen would have similar rewarding effects as muscimol and evaluated this hypothesis using intracranial self-administration and conditioned place preference procedures. In addition, we examined possible self-administration effects of baclofen into the posterior part of the ventral tegmental area (VTA), including the central linear nucleus (CL), since we previously found that rats learn to self-administer muscimol into the vicinity of the CL (Ikemoto et al. 1998). Our findings have important implications for use of baclofen in clinical treatments including affective disorders, drug addiction, and withdrawal (Heilig and Egli 2006; Kreek et al. 2002; Leggio et al. 2008; Sofuoglu and Kosten 2005).

Materials and methods

Animals

We used 225 male Wistar rats (Harlan, Dublin, VA, USA) weighing 250–350 g at the time of surgery. The colony room was maintained at consistent temperature and humidity on a reverse 12:12-h dark–light cycle (8:00_{A.M.} off). Food and water were freely available except during testing. All procedures were approved by the Animal Care and Use Committee of the Intramural Research Program at National Institute on Drug Abuse and were in accordance with the National Institutes of Health guidelines. The numbers of rats used in the experiments described below are reported on figures or in figure legends.

Surgery

Rats were stereotaxically implanted with permanent unilateral guide cannulae (24 gauge) under sodium pentobarbital (31 mg/kg, i.p.) and chloral hydrate (142 mg/kg, i.p.) anesthesia. Each rat's guide cannula ended 1.0 mm above one of the target regions. All cannulae were inserted into the left hemisphere at a lateral angle (0°, 6°, 10°, or 20°) toward the midline. The incisor bar was set at 3.3 mm below the interaural line. Stereotaxic coordinates (in millimeters) were 7.4 posterior to bregma (P), 1.6 lateral to the midline (L), and 8.0 ventral to the skull surface (V) angled at 10° for the MR; 7.2 P, 2.6 L, and 5.7 V with a 20° for the DR; 5.4 P, 1.9 L, and 8.0 V with a 10° for the VTA; and 6.3 P, 0.9 L, and 6.8 V with a 6° for the CL. Additional cannulae placements for control injection sites were aimed at the lateral DR (7.2 P, 2.9 L, and 5.5 V with a 20°); ventral DR (7.2 P, 2.7 L, and 6.2 V with a 20°), and pontine nucleus (7.4 P, 1.0 L, and 8.0 V with no angle). Each cannula was subsequently anchored to the skull by four stainless steel screws and dental acrylic and was inserted with a stainless steel wire (31 gauge) to keep it patent. Rats were housed singly to prevent other rats from chewing the implant after the surgery, which was followed by a minimum of 5 days of recovery before the start of experimentation.

Drugs

(*R*)-Baclofen and the GABA_B receptor antagonist SCH 50911 (Tocris Bioscience, Ellisville, MO, USA) were dissolved in artificial cerebrospinal fluid consisting of (in millimolars) 148 NaCl, 2.7 KCl, 1.2 CaCl₂, and 0.85 MgCl₂, pH adjusted to 6.5–7.5. The D1 receptor antagonist, SCH 23390 (Sigma-Aldrich, St. Louis, MO, USA), was dissolved in 0.9% sterile saline prior to intraperitoneal injection.

Experimental apparatus and self-administration procedure

Each rat was placed individually in an operant conditioning chamber (30×22×24 cm; Med Associates, St. Albans, VT, USA) equipped with two retractable levers (45 mm wide×2 mm thick, protruding 20 mm from the wall) located below cue lights on a side wall. An injection cannula was inserted and secured into the guide cannula, which was connected by polyethylene tubing to a micropump consisting of a drug reservoir and step motor (Ikemoto and Sharpe 2001) that hung a few millimeters above the rat's head. When activated, the micropump's step motor turned its shaft in eight incremental steps (9° per step) over 5 s, driving its threaded shaft into the drug reservoir and, in turn, pushing a 100- μ l volume out of the reservoir into the brain. Each session lasted 90 min or until the rats received a total of 60 infusions. The maximum number of infusions was set in order to minimize the possible tissue damage and drug diffusion. Sessions were separated by 24 h. One day before the start of testing, rats were placed in testing chambers for 90 min to habituate.

Experiment 1: self-administration as a function of brain site and baclofen concentration

We evaluated the effects of baclofen infusions into various midbrain regions on self-administration using the following single-lever procedure. A response on the lever (only one of the two levers in the apparatus was made available) retracted it and triggered an infusion (i.e., a fixed ratio 1 (FR1) schedule), the presentation of a 5-s tone, and the extinction of the light just above the lever. The lever and light cue were reinstated after a 15-s timeout. Each rat received vehicle in session 1 and 0.5 mM baclofen in sessions 2–4, to examine possible differential acquisition of self-administration between regions (acquisition phase). In sessions 5–8, each rat received vehicle, 0.1, 0.5, and 2.5 mM baclofen in this order, to examine the relationship between concentration and self-administration rate. These infusions were delivered into the DR, MR, VTA, CL, or sites just outside these regions. For a site-effectiveness analysis, we defined “effectiveness” of baclofen self-administration for each

rat as the second greatest rate among its self-administration sessions and plotted each injection site with its level of effectiveness on drawings.

Experiment 2: two-lever discrimination

We examined whether experimentally naïve rats would learn to discriminate the active lever, which administered baclofen into the DR or MR, from the inactive lever, which did not. Each rat was placed in the operant conditioning chamber described above with two levers made available. A response on the active lever retracted both levers and turned on the pump (i.e., a FR1 schedule), a tone, and the cue light above the lever for 5 s. The levers were reinstated after a 9-s timeout. A response on the inactive lever retracted both levers for 9 s, but did not lead to infusions or cue presentation. The left–right locations of the active and inactive levers were counterbalanced among rats, and the assignment of active and inactive functions between the levers remained the same for each rat throughout the experiment. Responding on the active lever resulted in vehicle infusions in session 1 and 0.5 mM baclofen in sessions 2–4.

Experiment 3: GABA_B receptors and baclofen self-administration

Using the GABA_B receptor antagonist SCH 50911, we examined whether GABA_B receptors mediate self-administration of baclofen into the MR and DR. In this experiment, MR and DR groups of the rats used in experiment 1 underwent the same instrumental procedure. Using a within-subjects design, they received vehicle, 0.5 mM baclofen alone, and a mixture of 1 mM SCH 50911 and 0.5 mM baclofen over three consecutive sessions. The order of these treatments was counterbalanced among the rats.

Experiment 4: dopamine-dependent effects of baclofen

We examined the effects of the D1 receptor antagonist SCH 23390 on self-administration of baclofen into the MR and DR. The rats that completed experiment 3 were treated with 0.9% saline (1 ml/kg, i.p.) 30 min before the start of session 1 and received vehicle infusions using the instrumental procedure described in experiment 1. In sessions 2 and 3, the rats received 0.5 mM baclofen. Thirty minutes before these sessions, the rats were treated with saline and SCH 23390 (0.025 mg/kg, i.p.). The order of these pretreatments was counterbalanced among the rats.

Experiment 5: conditioned place preference

Experimentally naïve rats were used for this experiment. The place conditioning chamber consisted of two compartments (21×21×28 cm³) linked by a connecting area (21×21×12.5 cm³); a sliding door separated each compartment from the connecting area (Med Associates). The two compartments differed in wall color (black vs. white), floor type (net vs. grid), and lighting; the amount of light was adjusted so that the rats did not prefer one compartment over the other prior to place conditioning. We use an “unbiased” procedure, in which drug and vehicle injections were randomly assigned between the two compartments without consideration of rats' original place preference. On day 1, each rat was placed in the connecting area of the chamber without any treatment; the rat had access to both compartments for 15 min, and the time spent in each compartment was recorded, using six pairs of infrared photobeam detectors in each compartment. On days 2–5, each rat received two sessions each day: one starting at 9:00_{A.M.} and the other starting at 3:00_{P.M.} Just before each session, each rat received an intracranial injection (300 nl delivered over 60 s) of either vehicle or baclofen (0.1 or 0.5 mM) into the DR or MR. An additional 30-s period elapsed before removal of the injection cannula. Following the injection, the rat was placed in one of the compartments and remained there for 30 min. The injections of vehicle and baclofen alternated over two sessions; the order of the injection treatments and the assignment of the

two compartments with these sessions were counterbalanced among the rats. On day6, each rat was placed in the connecting area of the chamber without any injection and had access to both compartments for 15 min; the time spent in each compartment was recorded. The chambers were wiped clean with a Nolvasan solution following each test or conditioning phase. Place preference score of each test was derived by subtracting the time spent in its vehicle compartment from the time spent in its drug compartment.

Histology

Upon completion of the experiments, the rats' brains were removed under deep pentobarbital (31 mg/kg, i.p.) and chloral hydrate (142 mg/kg, i.p.) anesthesia. The brains were placed in 10% formalin solution for a minimum of 2 days prior to sectioning on a cryostat. Frozen coronal sections (40µm thickness) near the cannula tip were mounted on gelatinized glass slides and stained with cresyl violet. The placements of injection cannulae were verified by microscopic examination.

Statistical analyses

Data were analyzed with the analysis of variance (ANOVA)/multivariate analysis of variance (MANOVA) module of Statistica (version 6.1, StatSoft, Inc., Tulsa, OK, USA). When the sphericity assumption examined by Mauchley sphericity test was violated for repeated factors, effects of the repeated factors were analyzed by MANOVAs; otherwise, ANOVAs were used. When factors with more than two levels were found to be significant, we performed Newman–Keuls post hoc tests.

Results

Experiment 1: self-administration as a function of brain site and baclofen concentration

Figure 1 shows photomicrographs of injection cannula tips of representative rats, and Fig. 2 shows the placements of injection cannulae tips for all rats that completed the testing in experiment 1 and summarizes the self-administration effectiveness of each injection site. The posterior portion (at 7.9, 8.2, and 8.5) of the MR tended to support the most vigorous self-administration of baclofen. Baclofen injections into the VTA or CL did not clearly increase self-administration (higher than 0.4 infusions per minute). Effects of intra-DR baclofen on self-administration varied.

Figure 3 depicts the data sorted by session and group. Rats quickly learned to self-administer baclofen into the MR. We did not observe differential responding as a function of concentration for intra-MR baclofen. Overall, regardless the concentration, rats self-administered baclofen at the mean rate of 0.4 infusions per minute (the total volume of 3.6µl per 90-min session). Our examination of event records did not show appreciable self-administration pattern associated with concentration and suggested that each rat responded uniquely to each concentration of intra-MR baclofen. Baclofen infusions into other brain regions did not support clear self-administration, although the DR or CL tended to slightly increase responding with baclofen. These observations were confirmed by a significant region×session interaction ($F_{12, 381}=6.66$, $P<0.01$) as well as a significant main region effect ($F_{3, 144}=13.92$, $P<0.01$; a 5×4 (region×session) mixed ANOVA/MANOVA on infusion rates). MR self-administration rates were significantly greater than those of any other regions with sessions collapsed together ($P<0.01$). Peculiarly, baclofen self-administration into the MR and DR tended to decrease over sessions (2–4) when the concentration was kept constant, possibly suggesting a development of tolerance for the drug. All concentrations of baclofen injections into the MR (sessions 6–8) supported self-administration (a significant region×concentration interaction ($F_{12, 379}=3.01$, $P<0.01$ with a 5×4 (region×concentration) mixed ANOVA/MANOVA on infusion rates). Administration of baclofen at any

concentration had no reliable effect in any other region. Again, MR self-administration rates were significantly greater than those of any other regions with sessions collapsed together ($P<0.01$; a significant main region effect, $F_{3, 143}=10.26$, $P<0.01$).

Experiment 2: two-lever discrimination effects

This experiment had two aims: one to address effects of baclofen-induced motor activity on lever pressing and the other to reexamine the possible rewarding effects of baclofen injections into the DR. To determine whether baclofen administration merely increased motor activity, we used a two-lever procedure to examine whether rats discriminated the active lever, which contingently delivered baclofen infusions, from the inactive lever, which did not. Since the data from experiment 1 did not show clearly whether rats self-administered baclofen into DR (its effects may have been so small that they could not be detected by the mixed-design analysis including four other groups), we examined the effects of baclofen injections into DR separately from those into the MR.

MR rats quickly learned to discriminate between the two levers and responded on the active lever more than the inactive lever during the acquisition phase (Fig. 4), a significant lever-by-session interaction ($F_{3, 36}=3.50$, $P<0.05$; a 2×4 (lever $\times$ session) repeated ANOVA/MANOVA on responses). Rats receiving baclofen into the DR increased responses (a significant session effect, $F_{3, 15}=8.94$, $P<0.01$). Although these rats responded on the active lever more than the inactive lever with session collapsed together (a significant lever effect, $F_{1, 17}=10.63$, $P<0.01$), they did not seem to change lever preference with or without baclofen, an observation that is reflected in a nonsignificant lever-by-session interaction.

Experiment 3: GABA_B receptors and baclofen self-administration

To verify whether GABA_B receptors mediate baclofen's self-administration effects, we examined the effects of coadministration of the GABA_B antagonist SCH 50911. Rats self-administered baclofen into the MR or DR at significantly lower rates when 1 mM SCH 50911 was coadministered (Fig. 5a; significant treatment effects, $F_{2, 32}=6.85$, $P<0.01$ and $F_{2, 36}=9.45$, $P<0.01$, respectively, after one-way repeated measures MANOVAs with three treatments on infusion rates).

Experiment 4: dopamine-dependent effects of baclofen

We examined whether baclofen self-administration into the MR and DR depends on intact dopamine systems by treating rats with the D1 receptor antagonist SCH 23390. Pretreatment with a low dose of SCH 23390 (0.025 mg/kg, i.p.) reduced rates of baclofen self-administration into the MR and DR (Fig. 5b; significant treatment effects $F_{2, 32}=9.26$, $P<0.01$ and $F_{2, 66}=14.70$, $P<0.01$, respectively, after one-way repeated measures ANOVA/MANOVAs, with three treatments on infusion rates).

Experiment 5: conditioned place preference

We tested whether baclofen infused into the DR or MR would induce conditioned place preference. We compared place preference scores between pre- and postconditioning and between the two regions (MR and DR), using 2×2 (conditioning $\times$ region) mixed ANOVA/MANOVAs. The low concentration of baclofen (0.1 mM) injected into the MR or DR tended to induce conditioned place preference (Fig. 6), although the effects were not reliable. Injections of 0.5 mM baclofen led to a reliable conditioned place preference ($F_{1, 20}=27.39$, $P<0.01$; Fig. 6), with regions collapsed together. DR injections induced more robust conditioned place preference than MR injections ($F_{1, 20}=4.74$, $P<0.05$; there was a strong trend for the interaction between region and conditioning, though it was not statistically significant).

Discussion

As we hypothesized on the basis of the rewarding effects of muscimol administered into the MR, we found that baclofen was self-administered into the MR. Although we found differences between vehicle and baclofen infusions, we failed to detect any concentration effect on responding. In a typical intracranial self-administration study, we observe an ascending relationship between response rate and concentration. In our previous studies, we rarely observed inverted “U”-curved relationships between response and concentration (e.g., see Ikemoto and Wise 2002) possibly because intracranially administered drugs do not stay long at the injection or reward-responsive site and diffuse quickly. In this case, we speculate that rewarding effects of baclofen are weak compared to those of other manipulations we have studied, leading to a small window to detect difference between concentrations due to a ceiling effect. If we had tried lower concentrations, we would have seen an ascending response–concentration relationship for baclofen. Baclofen self-administration into the MR was attenuated by coadministration of the GABA_B receptor antagonist SCH 50911, suggesting that baclofen self-administration is mediated by GABA_B receptors.

We found differential effects of baclofen on self-administration between the MR and DR. Baclofen self-administration into the DR was so slight that we detected it only after testing many subjects for a within-subjects analysis. Similar to intra-DR baclofen, intra-CL baclofen had a slight effect in experiment 1. Because we did not conduct additional experiment for the CL, future research needs to determine if intra-CL baclofen is rewarding.

Baclofen administration into the midbrain raphe nuclei may recruit the activation of the mesolimbic dopamine system

Although it is not well documented, the midbrain raphe nuclei may interact with the mesolimbic dopamine system. First of all, midbrain raphe neurons send efferent projections to the VTA, ventral striatum, and receive afferents from the ventral striatum, through the ventral pallidum, and the VTA (Behzadi et al. 1990; Geisler and Zahm 2005; Marcinkiewicz et al. 1989; Vertes 1991; Vertes and Martin 1988). Self-administration of baclofen depends on intact dopamine transmission since it was readily decreased by a low dose of systemic administration of dopamine receptor antagonists; similar effects of dopamine receptor antagonists are also found on muscimol self-administration into the MR (Liu and Ikemoto 2007). While it has not yet been determined if intra-MR baclofen injections increase dopamine release in the ventral striatum, it is shown that intra-MR muscimol injections increase the ratios of DOPAC or HVA to dopamine in postmortem accumbens tissues (Wirtshafter and Trifunovic 1992), suggesting that this manipulation increase extracellular dopamine levels in the nucleus accumbens.

Issues of interpreting self-administration data

Rats self-administered baclofen into the MR at rates of 0.4/min or more, receiving total volumes of 3.5µl or more over the 90-min session. Although intra-MR baclofen infusions may have affected a large area, actual trigger zone for baclofen reward may not be much different than what is characterized in Fig. 2. Rats self-administered much less when they received vehicle (about 1µl over the course of 90 min) or when the drug is delivered nonreinforcing sites. Only when the drug is rewarding, rats self-administer the drug more, possibly affecting more area. Although we do not know how baclofen diffused when rats delivered a total of 3.5µl or more over the course of the session, it is reasonable to conclude that baclofen is self-administered when infused into the vicinity of the posterior MR.

Self-administration of baclofen in experiment 1 was assessed using a single lever procedure. This procedure does not rule out the interpretation that increased lever pressing is due to

baclofen's effects on motor activity or "general" arousal. To address this issue, we conducted the two lever experiment, which found that rats receiving baclofen into the MR respond more on the active lever, which delivered baclofen, than the inactive lever. Although we also observed that baclofen injections into the MR increased inactive lever pressing, this observation is probably not due to mere general arousal since in another study (Shin et al. 2010), we observed that a high dose of amphetamine injection (3 mg/kg, i.p.) that markedly increased locomotion did not increase lever pressing at all in the same chambers that we used here. In any case, two lever data reject the interpretation that MR injections of baclofen were without motivational effects. We cannot state the same for baclofen injections into the DR because intra-DR rats did not selectively increase responding on the active lever. However, positive reinforcing effect of baclofen injections into the DR was detected by our conditioned place preference data.

Baclofen administration into the MR or DR induced conditioned place preference

Place conditioning procedures can demonstrate reinforcement without confound of motor effects. Conditioned place preference depends on recalling associative memory between environmental stimuli present in the drug-paired compartment and affective arousal induced by drug injections. The fact that place conditioning testing was performed with the absence of baclofen excludes any concern for motor/general arousal effects. Baclofen injections into the DR induced clear conditioned place preference, while baclofen injection into the MR induced less robust preference. These findings suggest that baclofen administration into the midbrain raphe nuclei elicits positive affective arousal, which leads to stimulus-reward associative learning.

It is difficult to interpret the finding that baclofen administration into the DR induced conditioned place preference more effectively than administration into the MR in light of the finding that baclofen is self-administered more effectively into the MR than into the DR. These results suggest that both MR and DR are involved in reward but that the two sites are involved differently. It is tempting to suggest that the DR is more importantly involved in place conditioning processing, while the MR in self-administration processing. This hypothesis is consistent with the previous finding that 8-OH-DPAT injections into the DR induce conditioned place preference at a ten times lower dose than injections into the MR (Fletcher et al. 1993). However, it is difficult to reconcile with our previous finding that MR injections of the GABA_A receptor agonist muscimol are more effective in both supporting self-administration and inducing conditioned place preference than DR injections (Liu and Ikemoto 2007). Although it is unclear at this time why the MR and DR appear to be involved in baclofen reward differently, the differential effects between baclofen and muscimol on reward may partly depend on how these manipulations influence serotonergic and nonserotonergic neurons, a topic that is discussed in the next section.

Differential effects of muscimol and baclofen injections into the midbrain raphe nuclei may be explained by differential responses of serotonergic and nonserotonergic neurons

GABA_A receptors within the MR and DR are expressed on both serotonergic and nonserotonergic neurons (Moore 1981). Although stimulation of GABA_A receptors in the MR or DR can inhibit serotonergic neurons and reduce extracellular serotonin in the forebrain (Judge et al. 2004; Shim et al. 1997), behavioral effects elicited by stimulation of GABA_A receptors in these regions appear to be importantly mediated by nonserotonergic neurons. Selective lesions of serotonergic neurons by intra-MR 5,7-DHT or serotonin depletion by systemic *p*-chlorophenylalanine do not affect locomotor activity elicited by muscimol injections into the MR (Fink and Morgenstern 1986; Paris and Lorens 1987; Wirtshafter et al. 1987). These findings suggest that nonserotonergic neurons play an important role in mediating behavioral effects of intraraphe muscimol.

GABA_B receptors appear to be localized on serotonergic neurons, but not on nonserotonergic local neurons in the MR or DR (Serrats et al. 2003; Varga et al. 2002; Wirtshafter and Sheppard 2001). This structural information is consistent with the finding that stimulation of GABA_B receptors in the MR or DR inhibits serotonergic neurons (Colmers and Williams 1988; Innis and Aghajanian 1987; Tao et al. 1996). However, GABA_B receptors are also found on the terminals of GABAergic afferents, which can inhibit tonic GABAergic inputs to serotonergic neurons, thereby disinhibit them (Abellán et al. 2000b). Therefore, baclofen injections into the MR or DR could have opposing effects on serotonergic neurons. Intra-DR application of baclofen, particularly at high concentrations, is found to reduce extracellular serotonin concentrations in the dorsal striatum (Abellán et al. 2000b). It is possible that intraraphe baclofen, particularly at low concentrations, increases serotonin release in the forebrain, although such effects have not yet been demonstrated. In summary, these findings suggest that injections of baclofen and muscimol into the DR or MR may differentially impact on serotonergic and nonserotonergic neurons, thereby differential impact on reward processes.

Midbrain serotonergic neurons and affective processes

Roles of serotonergic neurotransmission in affective processes may qualitatively differ between minutes and weeks after alteration. Brain serotonin has been implicated in pathophysiology and treatments of major depression. Most of effective treatments for major depression are associated with enhancement of brain serotonin neurotransmission (Blier and de Montigny 1994). Consistently, depressed patients appear to have reduced central serotonergic activity (Coccaro et al. 1989). However, the relationship between serotonergic transmission and remission of mood disorders is not simple. Antidepressants such as selective serotonin reuptake inhibitors increase levels of extracellular serotonin in a few minutes, whereas remission emerges only after a few weeks of continuous treatments. In the present study, injections of baclofen into the midbrain raphe nuclei, which most likely inhibited serotonergic neurons and reduced forebrain serotonin, appear to have elicited a positive affective state and arousal, effects that are akin to mania rather than depression.

Clinical implications

Baclofen and other GABA_B receptor agonists are being considered for the treatments of drug and alcohol dependence (Cousins et al. 2002; Heilig and Egli 2006; Leggio et al. 2008) and mania (Emrich et al. 1980; Krupitsky et al. 1993; Taylor et al. 2003). In light of our finding, clinical trials should be carried out with special attention to individual differences in response to these drugs. Indeed, several papers reported that rather than attenuation of mania, high dose of oral baclofen induced manic-like states accompanied by euphoria (Stewart 1992; Wolf et al. 1982; Yassa and Iskandar 1988).

In conclusion, our findings suggest that stimulation of GABA_B receptors in the midbrain raphe regions is rewarding and support the view that inhibition of midbrain raphe neurons leads to disinhibiting reward-related processes.

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Fig. 1.
Photomicrographs of the tips of injection cannulae. Representative placements of injection tips are shown by *arrows* for the median (**a**) and dorsal (**b**) raphe nuclei

Fig. 2.

Coronal sections showing injection sites and their effectiveness at inciting self-administration. The ventral tip (0.4 mm) of each injection cannula ($N=151$) is color-coded to indicate the effectiveness of baclofen self-administration. Effectiveness was derived by obtaining the second greatest rate (the greatest rates seem to vary and less reliable) of baclofen self-administration among the sessions. The *number* in each brain section indicates the distance from bregma. The brain sections were adapted from the atlas of (Paxinos and Watson 2005). *CL* central linear nucleus, *DR* dorsal raphe nucleus, *MR* median raphe nucleus, *VTA* ventral tegmental area

Fig. 3.

Self-administration of baclofen over sessions as a function of brain region. Each injection site shown in Fig. 1 was grouped into one of five regions: median raphe nucleus (*MR*; $N=47$), dorsal raphe nucleus (*DR*; $N=38$), central linear nucleus (*CL*; $N=9$), ventral tegmental area (*VTA*; $N=17$), and sites outside of regions of interest (*OUT*; $N=40$). Data are mean self-administration rates ($\pm$ SEM). * $P < 0.01$, significantly greater from its vehicle value

Fig. 4.

Self-administration of baclofen with a two-lever procedure. Data ($N=13$ for MR; $N=18$ for DR) are mean lever presses ($\pm$ SEM). * $P<0.05$, significantly greater from vehicle values with lever collapsed together. # $P<0.01$, significantly greater than its inactive lever value and its vehicle active lever value. % $P<0.05$, significantly greater from its vehicle inactive lever value

Fig. 5.

Disruption of baclofen self-administration by the blockade of GABA_B receptors or dopamine receptors. **a** Effects of the GABA_B receptor antagonist SCH 50911 are shown ($N=34$ for MR and $N=38$ for DR). **b** Effects of the dopamine receptor antagonist SCH 23390 are shown ($N=34$ for MR and $N=34$ for DR). * $P<0.01$, significantly greater from the other two treatments

Fig. 6.
Conditioned place preference induced by baclofen administration into the MR or DR.
* $P < 0.05$, greater than MR values