The progesterone metabolite allopregnanolone potentiates $GABA_A$ receptor-mediated inhibition of 5-HT neuronal activity

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Abstract

The dorsal raphe nucleus (DRN) is the origin of much of the 5-HT innervation of the forebrain. The activity of DRN 5-HT neurons is regulated by a number of receptors including $GABA_A$ and $5\text{-}HT_{1A}$ inhibitory receptors and by excitatory α_1-adrenoceptors. Using *in vitro* electrophysiological recording we investigated the action of progesterone and its metabolite, allopregnanolone on receptor-mediated responses of DRN 5-HT neurons. Neither allopregnanolone nor progesterone affected the α_1-adrenoceptor agonist-induced firing. Allopregnanolone also had no effect on the inhibitory response to 5-HT. However, allopregnanolone significantly potentiated the inhibitory responses to $GABA_A$ receptor agonists. Progesterone did not enhance $GABA_A$ receptor-meditated inhibitory responses. Thus, the neuroactive metabolite of progesterone, allopregnanolone, has the ability to cause potentiation of $GABA_A$-mediated inhibition of DRN 5-HT neurons. This effect on 5-HT neurotransmission may have relevance for mood disorders commonly associated with reproductive hormone events, such as premenstrual dysphoric disorder and postpartum depression.

Keywords: electrophysiology, neurosteroid, rat, serotonin, slice.

Introduction

In both rat and man the majority of 5-HT projections in the brain originate from neurons in the dorsal raphe nucleus (DRN) (Azmitia and Gannon, 1986; Dahlström and Fuxe, 1964; Tork, 1985). The activity of these DRN neurons, and the consequent 5-HT release from their forebrain terminals, is controlled by a number of excitatory and inhibitory receptors (Piñeyro and Blier, 1999). These include α_1-adrenoceptors which provide the main excitatory drive (VanderMaelen and Aghajanian, 1983) and $5\text{-}HT_{1A}$ inhibitory autoreceptors, which inhibit firing (Sprouse and Aghajanian, 1987). In addition, GABA has been shown to play an important role in the control of 5-HT neurons. Thus, $GABA_A$ receptor activation inhibits 5-HT neuronal firing in the DRN (Gallager, 1978; Judge et al., 2004a) and 5-HT release in the forebrain (Tao et al., 1996). Moreover, there is evidence for significant $GABA_A$ receptor-mediated tone on 5-HT neuronal firing and release *in vivo* (Gervasoni et al., 2000; Levine and Jacobs, 1992).

5-HT is involved in many physiological functions (Jacobs et al., 1990; Jacobs and Fornal, 1991) and dysfunction of 5-HT neurotransmission is thought to underlie mood disorders (see Meltzer, 1989). It is well established that mood disorders are more prevalent in women than in men (Kessler et al., 1993), suggesting a role for gonadal steroids in their aetiology. This is further supported by the fact that mood disorders are commonly associated with reproductive hormone events such as the menstrual cycle, pregnancy and the postpartum period (Yonkers et al., 2003). Interestingly, changes in gonadal steroid hormone levels have been shown to affect aspects of 5-HT

neurotransmission (Bouali et al., 2003; Lu and Bethea 2002), thus providing a mechanism by which reproductive hormone events may lead to changes in mood.

In addition to oestrogen and progesterone, levels of the progesterone metabolite, allopregnanolone (3α-hydroxy-5α-pregnan-20-one; 3α,5α-tetrahydroprogesterone) vary over reproductive hormone events (Girdler et al. 2001; Hill et al., 2001; Wang et al. 1996). Allopregnanolone has been shown to positively modulate $GABA_A$ receptor-evoked responses in hippocampal and cortical neurons (Mtchedlishvili et al., 2003; Puia et al., 1993; Stell et al., 2003). To date there are few data on the effects of neuroactive steroids on serotonergic neurons. However, a recent report showed that i.c.v. treatment of female rats with allopregnanolone increased the firing rate of DRN neurons recorded *in vivo* (Robichaud and Debonnel, 2004).

The aim of this present study was to examine whether allopregnanolone alters the receptor-mediated regulation of 5-HT neurons in the DRN. *In vitro* electrophysiology was used to determine if allopregnanolone modulated responses to agonists of $GABA_A$ receptors and $5\text{-}HT_{1A}$ autoreceptors, or affected α_1-adrenoceptor driven activity.

Method

Experimental Animals

All experiments had been reviewed and approved by the University of Newcastle Ethical Review Panel and were conducted in accordance with the UK Animals (Scientific Procedures) Act of 1986 and the European Community Council Directive

(86/609/EEC). All efforts were made to minimize any pain or discomfort of the animals. Adult female Hooded-Lister rats (200-300g body weight; Charles River, Kent, UK) were group housed under controlled conditions of temperature (22 ± 2 °C) and humidity (40%) in a 12 h light/dark cycle (lights on 7 am). All animals were allowed to acclimatise to the holding facilities for at least one week before use. Animals, selected at random stages of the oestrous cycle, were decapitated and trunk blood collected.

Electrophysiology

Following decapitation the brain was quickly removed and submerged in oxygenated (95% O_2-5% CO_2) sucrose slush (composition (mM): sucrose 200, HEPES 10, $MgSO_4$ 7, NaH_2PO_4 1.2, KCl 2.5, $NaHCO_3$ 25, $CaCl_2$ 0.5, D-glucose 10, pH 7.4) for 5 min. The caudal portion of the brain was then mounted on a block using cyanoacrylate glue and submerged in oxygenated sucrose slush in the chamber of a vibrating microtome (Vibratome 1000, Vibratome, St. Louis, USA). Coronal slices (> 3) of the midbrain (400 μm thick) containing the DRN were cut and transferred to a Petri dish containing oxygenated artificial cerebrospinal fluid (aCSF) (composition (mM): NaCl 124, $MgSO_4$ 2.4, KH_2PO_4 1.25, KCl 3.25, $NaHCO_3$ 26, $CaCl_2$ 2, D-glucose 10, pH 7.4). Slices between bregma -7.6 and -8.3 mm (Paxinos and Watson, 1998) were trimmed to remove cortical tissue and one slice was transferred to an interface perfusion chamber and perfused with aCSF (0.5 ml/min) at 36˚C. Slices were allowed to equilibrate for at least 30 min prior to recording. The remaining slices were maintained in oxygenated aCSF at room temperature until required. The aCSF used during recording contained the α_1-adrenoceptor agonist phenylephrine (1 μM) in order to evoke sustained 5-HT neuronal activity.

Extracellular recordings were made from presumed 5-HT neurons in the DRN using glass microelectrodes (1.5 mm OD, Clarke Electromedical, Reading, UK; 1-3 MΩ *in vitro* impedance) filled with 2 M NaCl. Signals were amplified (x1000) with an AC differential preamplifier and then fed to a pulse discriminator. The TTL pulses generated were fed to the PC via a computer interface (1401 or micro1401, CED, Cambridge, UK) at a sampling rate of 100 Hz. Data were collected using Spike2 software (version 4, CED, Cambridge, UK). The microelectrode was advanced slowly through the slice (approximately 0.15 mm/min) to decrease the probability of missing neurons with slow firing rates. Neurons were identified as putative 5-HT neurons on the basis of their location (in the DRN), basal electrophysiological characteristics (regular single spikes 1-2 ms wide with a slow frequency (0.4-3.5 Hz)) (Allers and Sharp, 2003; VanderMaelen and Aghajanian, 1983), and their inhibitory response to 5-HT (10-100 μM). Following a period of recording of basal firing activity, drugs were applied via the perfusion medium at intervals of at least 4 min. 5-HT and the $GABA_A$ receptor agonists, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol hydrochloride (THIP; Gaboxadol) and muscimol, were applied for periods of 2 min. The concentration of $GABA_A$ receptor agonists producing a submaximal response was determined for each neuron. This concentration was used for subsequent applications. Allopregnanolone and progesterone were applied for periods of 3 or 8 min before and 2 min during reapplication of the $GABA_A$ receptor agonist. The agonist was then applied at intervals after the steroid application (10, 30, 60, 90 min) for as long as the recording was stable. Due to potential irreversible effects, allopregnanolone or progesterone were only applied once to each slice, unless

otherwise stated. Therefore only one neuron was recorded from each slice and between one and three neurons were recorded from each animal.

Materials

NaCl, $MgSO_4$, KH_2PO_4, KCl, $NaHCO_3$ and sucrose were purchased from BDH Laboratory Supplies. $CaCl_2$, D-(+)-glucose, HEPES, muscimol, L-phenylephrine hydrochloride, progesterone and 5-HT hydrochloride were all purchased from Sigma. THIP was purchased from Tocris. Allopregnanolone was purchased from Merck Biosciences Ltd. Allopregnanolone and progesterone were dissolved in ethanol before diluting in aCSF, resulting in a final maximum ethanol concentration of 0.025%. Other drugs were dissolved in aCSF.

Data analysis and statistics

Electrophysiological data were analysed offline. Basal firing was determined in a 120 s period at the start of the recording and expressed in Hz. A response to drug application was defined as a greater than 5% change from basal over the period of application of the drug. Inhibitory responses were determined as the firing rate in a 120 s period covering the maximum change in baseline and expressed as a percentage change relative to the firing rate in the 120 s immediately before drug application. The 120s period was chosen as this was the length of time the slice was perfused with the agonist. Data presented are individual examples or the mean ± SEM. Differences were analysed by paired t-test or repeated measures ANOVA with *post hoc* paired t-test analysis.

Serum sample analysis

Trunk blood collected at the time of decapitation was allowed to clot on ice before samples were centrifuged. The resulting serum was removed and stored at -20°C until assayed. Samples from 16 animals were assayed for the ovarian sex steroids, progesterone and oestradiol using commercially available radioimmunoassay kits (immuchem^{TM} coated tube, MP Biomedicals, USA and Diagnostic Systems Laboratories, USA, respectively).

Results

In the presence of the α_1-adrenoceptor agonist phenylephrine (1 μM) presumed 5-HT neurons in the DRN fired slowly and regularly (mean basal firing rate: 1.04 ± 0.06 Hz; range 0.43 – 2.07 Hz; *n* = 44 neurons from 29 animals) (Fig. 1A). Application of 5-HT (10-100 μM) inhibited all neurons in a fully reversible manner (mean inhibition by 25 μM 5-HT: 70.2 ± 5.4%; *n* = 36) (e.g. Fig. 1A).

The $GABA_A$ receptor agonist THIP (2 min application) caused a concentration-dependent decrease in the firing rate of presumed 5-HT neurons (Fig. 1A). The THIP response was not related to the stage of oestrus at the time the tissue was taken. Thus, there was no correlation between the inhibitory responses to THIP (25 μM) and the serum levels of either progesterone or oestradiol of individual animals (progesterone: r = -0.31, p = 0.24; oestradiol: r = 0.15, p = 0.59; Pearson correlation, *n* = 16 animals, data not shown).

The effect of allopregnanolone (5 μM) was tested in eleven neurons (nine animals). Allopregnanolone (5 μM) alone did not affect the basal firing rate after the 5 or 10 min application (firing rate before allopregnanolone: 0.96 ± 0.14 Hz; 10 min after allopregnanolone: 0.95 ± 0.12 Hz; 30 min after allopregnanolone: 1.03 ± 0.13 Hz; $F_{2,20} = 2.8$, $p = 0.08$, Repeated measures ANOVA). However, the inhibitory response to THIP was enhanced by allopregnanolone (Fig. 1A,B). This effect was rapid as the response to THIP obtained in the presence of allopregnanolone was potentiated. Further 2 min applications of THIP, 10 and 30 min after allopregnanolone application, revealed that the potentiation of the THIP response both persisted and increased with time (Fig. 1A,B; $F_{3,30} = 14.2$, $p < 0.001$, Repeated measures ANOVA). In three neurons the effect was followed for a prolonged time. The potentiation of the response to THIP was still observed up to 90 min after exposure to allopregnanolone (mean inhibition at 90 min: 100 ± 0 % vs. control response: 65.9 ± 9.8 %). Due to this persistent effect only one neuron was examined in each slice.

In a further five neurons (four animals), the effect of 1 μM and 5 μM allopregnanolone was examined. Repeated measures ANOVA over the time course of the experiment revealed a significant within subjects effect ($F_{6,24} = 6.0$, $p<0.01$). Although the inhibitory response to THIP was not increased during or 10-30 min after the application of 1 μM allopregnanolone (Fig. 1C; *post-hoc* paired t-test), in this same group of neurons the response was significantly enhanced during, and 10 and 30 min after, the application of 5 μM allopregnanolone (Fig. 1C; $p<0.05$, *post-hoc* paired t test).

The possibility that the allopregnanolone-induced potentiation of the THIP response was due to the solvent (0.025% ethanol) or to repeated applications of THIP was examined in nine neurones before allopregnanolone or progesterone was applied. In contrast to the effect of allopregnanolone, the ethanol vehicle (0.025%) failed to enhance the response to a second application of THIP ($p = 0.41$, paired t-test; $n = 9$).

The inhibitory response to the potent $GABA_A$ receptor agonist muscimol (0.33 - 1 μM) was also significantly enhanced by allopregnanolone (Fig. 2A; $F_{6,18} = 7.8$, $p<0.001$, Repeated measures ANOVA; $n = 4$). Post hoc analysis showed significance 10 min after the application of 5 μM allopregnanolone (Fig. 2B; $p < 0.05$, *post-hoc* paired-t-test). The inhibitory response to muscimol was also significantly enhanced during the application of 1 μM allopregnanolone (Fig. 2B; $p < 0.05$, *post-hoc* paired-t-test).

In five neurons (three animals) the response to 5-HT was tested before and after allopregnanolone. While, the inhibitory response to THIP increased ($p < 0.05$, paired t-test; $n = 5$), allopregnanolone (5 μM) failed to enhance the inhibitory response to 5-HT ($p = 0.95$, paired t-test; $n = 5$) (Fig. 3).

The effect of a 5 min application of progesterone (5 μM) on the inhibitory response to THIP (10-25 μM) was also examined (Fig. 4). Progesterone alone had no effect on the basal firing rate (Fig. 4A; $p = 0.15$, paired t-test; $n = 5$). Likewise, the magnitude of the inhibitory response to THIP applied during and 10 min after the progesterone application was not different to the control inhibitory response to THIP (Fig. 4A,B; $F_{2,8} = 1.3$, $p = 0.33$, Repeated measures ANOVA; $n = 5$).

Discussion

The present study examined whether allopregnanolone or progesterone altered receptor-mediated regulation of 5-HT neurons in the DRN. Acute *in vitro* application of allopregnanolone enhanced the inhibitory response of 5-HT neurons to the $GABA_A$ receptor agonists, THIP and muscimol. However, allopregnanolone had no effect either on the inhibitory response to 5-HT or on the basal firing rate, which was evoked by α_1-adrenoceptor activation. In addition, acute application of progesterone had no effect on the $GABA_A$ response or on α_1-adrenoceptor-driven 5-HT neuronal firing. Our data show that allopregnanolone selectively enhances $GABA_A$ receptor regulation of 5-HT neuronal firing.

Identity of neurons recorded

The DRN neurons recorded in this study fired in a slow and regular pattern and were inhibited by 5-HT and so are equivalent to the population of neurons previously studied by a number of groups (Abellán et al., 2000; Hanoun et al., 2003; Liu et al., 2002), including our own (Fairchild et al., 2003; Johnson et al., 2002; Judge et al., 2004a,b). Although recent evidence suggests that some non-5-HT neurons in the DRN may display the same electrophysiological characteristics as 5-HT neurons and are inhibited by 5-HT (Allers and Sharp, 2003; Beck et al., 2004; Kirby et al., 2003), we are confident that the vast majority of neurons we recorded in this study are serotonergic (see Judge et al., 2004a).

Effect of allopregnanolone on receptor regulation of 5-HT neuronal activity

The selective $GABA_A$ receptor agonists, THIP and muscimol caused a clear inhibition of firing of all 5-HT neurons tested in this study. We have previously reported that the 5-HT neuronal responses to THIP are completely blocked by the $GABA_A$ receptor antagonist bicuculline (Judge et al., 2004a), indicating the action of THIP is mediated by $GABA_A$ receptors. In the present study, the $GABA_A$ receptor responses to both THIP and muscimol in 5-HT neurons were enhanced by allopregnanolone. This is consistent with previous studies which have shown the positive modulatory effects of allopregnanolone on the $GABA_A$ receptor responses of neurons of different subtypes and from other brain regions (Lambert et al., 1990, Puia et al., 1993). However, whilst allopregnanolone has been shown to modulate spontaneous or GABA-induced chloride currents (e.g. Fodor et al., 2005; Puia et al., 1993; Zhang and Jackson, 1994) or the amplitude of evoked population spikes in CA1 pyramidal neurons (e.g. Landgren et al., 1998), to our knowledge this is the first report of single unit activity modulation. This measure is a direct index of neuronal output and the ability of allopregnanolone to modulate the GABAergic response of 5-HT neurons will have major implications for serotonergic neurotransmission.

Interestingly, allopregnanolone alone did not inhibit DRN 5-HT neuronal firing. Several conclusions can be drawn from this observation. Firstly, the action of allopregnanolone is allosteric rather than direct. There is some evidence that allopregnanolone can directly activate $GABA_A$ receptors on cultured neurons, although the efficacy of allopregnanolone is much lower than that of GABA (Callachan et al., 1987; Lambert et al., 1990; Puia et al., 1993). Secondly, the failure of allopregnanolone to alter basal firing suggests that DRN 5-HT neurons in the slice preparation are not under tonic $GABA_A$ regulation. This is consistent with our

previous observation that the $GABA_A$ antagonist bicuculline alone does not increase 5-HT neuronal firing (Judge et al., 2004a). However, it should be noted that *in vivo* studies are indicative of significant $GABA_A$ receptor-mediated tone regulating 5-HT neuronal firing and release (Gervasoni et al., 2000; Levine and Jacobs, 1992). Finally, the α_1-adreonoceptors, which were tonically activated by phenylephrine in order to evoke 5-HT neuronal activity, are not modulated by allopregnanolone.

We have also demonstrated that allopregnanolone does not modulate the inhibitory response to 5-HT. As we have previously shown that under these recording conditions the response of DRN 5-HT neurons to 5-HT is mediated by $5\text{-}HT_{1A}$ receptors (Johnson et al., 2002), the data indicates that the effect of allopregnanolone for $GABA_A$ receptors is specific.

Progesterone, at a concentration equivalent to the highest concentration of allopregnanolone used, had no effect on either the basal firing rate of DRN 5-HT neurons or the response to $GABA_A$ receptor-activation. This suggests that a non-specific steroid effect (on membrane fluidity, for example) did not underlie the action of allopregnanolone. We also found that although ethanol is a known modulator of $GABA_A$ receptors (Davies et al., 2003), at the low concentration used here ethanol vehicle did not enhance the $GABA_A$ receptor response. Moreover, repeated application of THIP failed to potentiate the response of the 5-HT neurons. Taken together, these data indicate a specific enhancement of $GABA_A$ receptor mediated function by allopregnanolone.

The mechanism underlying the potentiation of the $GABA_A$-mediated responses by allopregnanolone is likely to be the well established allosteric modulation of the $GABA_A$ receptor, increasing the binding affinity of receptor agonists and enhancing chloride uptake and currents (Majewska, 1990; Zhang and Jackson, 1994). These effects are mediated through a site on the receptor distinct from the benzodiazepine and barbiturate allosteric sites (Lambert et al., 1990). In the slice preparation used in this study the potentiation persisted (and even increased with time) after a brief application of allopregnanolone. It is unclear if this is a temporal effect or indicates that the repeated $GABA_A$ receptor activation (with THIP or muscimol), is necessary to observe the potentiation. In our recording system the washout of non-steroidal compounds is very quick as evidenced by the profile of the responses to 5-HT and THIP (see Fig. 1A, 4A). However, lipophyllic compounds like allopregnanolone are likely to persist in the tissue for longer. Indeed, the effect of allopregnanolone on $GABA_A$ receptor activation has been previously described as slowly reversible (Poisbeau et al., 1997). Therefore, we may have seen a reversal in the long lasting potentiation if it had been possible to record longer than 90 min.

Interestingly, 1 μM allopregnanolone enhanced the response to muscimol but not to THIP. The explanation for this is unclear but it is unlikely to be due to differential subunit selectivity of the agonists. Thus, $GABA_A$ receptors containing the δ subunit are thought to be particularly sensitive to modulation by allopregnanolone (Stell et al., 2003) and THIP has highest affinity for this type while muscimol does not (Brown et al., 2002). From studies to date the subunit composition of the $GABA_A$ receptors on 5-HT neurones in the DRN is uncertain (Goa et al., 1993; Moragues et al., 2000; Rodriguez-Pallares et al., 2001).

As DRN 5-HT neurons are reportedly under tonic $GABA_A$ receptor inhibition *in vivo* (Gervasoni et al., 2000; Levine and Jacobs, 1992), the effects of allopregnanolone on $GABA_A$ receptor-mediated inhibition of firing will have consequences for 5-HT neurotransmission. In females, progesterone levels vary greatly during the various phases of reproductive life, and evidence suggests that brain levels of allopregnanolone mirror these changes (Wang et al., 1996). At times when allopregnanolone levels are high, $GABA_A$ receptor-mediated responses may be expected to be enhanced and 5-HT neuronal activity may be predicted to be greatly diminished. Consistent with this, a recent *in vivo* study has reported that $GABA_A$ receptor inhibition of 5-HT release varies over the oestrous cycle, being greatest during proestrus when progesterone, and hence allopregnanolone, levels are highest (Felton and Auerbach, 2004). Similarly, in women, a potential mechanism underlying the association between the affective state of premenstrual dysphoric disorder and the luteal phase rise in progesterone may include an allopregnanolone-mediated potentiation of GABAergic inhibition of serotonergic transmission. However, not all states of elevated progesterone are associated with decreased 5-HT level. Progesterone levels are at their highest during pregnancy and although changes in 5-HT neuronal firing rate have been observed, firing rates are reportedly raised rather than lowered during pregnancy (Klink et al., 2002). This apparent discrepancy may be explained by evidence from a number of studies, which indicates that chronic exposure (2-5 days) to allopregnanolone results in adaptive changes to $GABA_A$ receptors (Friedman et al., 1993; Yu and Ticku, 1995). Thus, during pregnancy when progesterone and allopregnanolone levels are raised for a sustained period, the enhancement of $GABA_A$ receptor function may be lost. This may also explain why

chronic allopregnanolone treatment (7 days) was shown to increase the firing rate of DRN neurons recorded *in vivo* (Robichaud and Debonnel, 2004).

In summary, the present data demonstrate that the progesterone metabolite, allopregnanolone, enhances the inhibitory response of 5-HT neurons to $GABA_A$ receptor activation. This may have implications for psychiatric disorders associated with changes in the levels of the ovarian steroid hormones.

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Statement of interest

None

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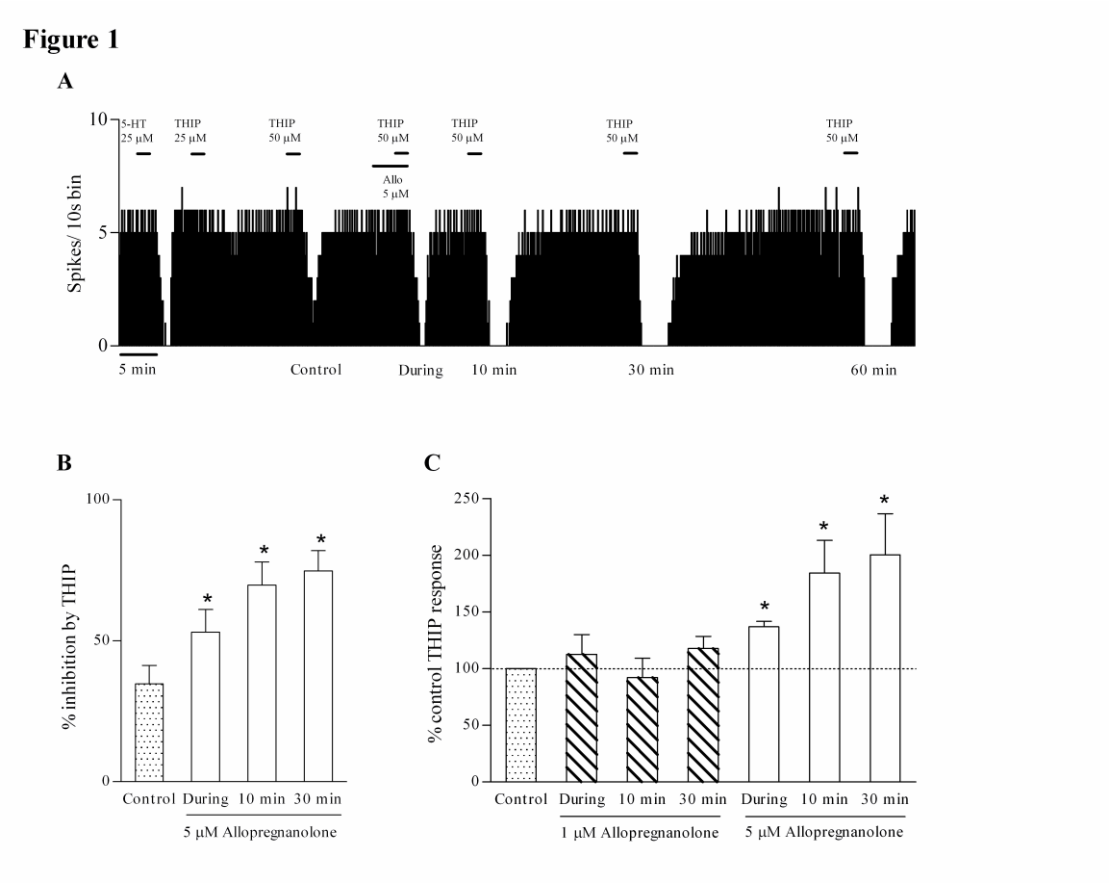

Figure 1. Enhancement of inhibitory responses to the $GABA_A$ agonist THIP by allopregnanolone. (A) Example of a recording from an individual 5-HT neuron in the DRN showing responses to THIP (50 μM) before (control), during and after (10 min, 30 min and 60 min) the application of allopregnanolone (Allo, 5 μM) for 5 min. Basal activity was recorded in the continuous presence of the α_1-adrenoceptor agonist, phenylephrine. THIP was applied for periods of 2 min and allopregnanolone was applied for 5 min as indicated by the bars. Note that recordings have not been corrected for the perfusion lag (approximately 2 min). (B) Mean percentage inhibition of 5-HT neuronal firing by THIP (25-50 μM) before (control), during and 30 min after application of allopregnanolone (n = 11). (C) Concentration-dependent effect of allopregnanolone (1 and 5 μM) on the potentiation of inhibitory responses to THIP. Data are shown as a percentage of the control response to THIP (n = 5) (Mean ± SEM). * $P < 0.05$, paired t-test vs. response prior to steroid application (control).

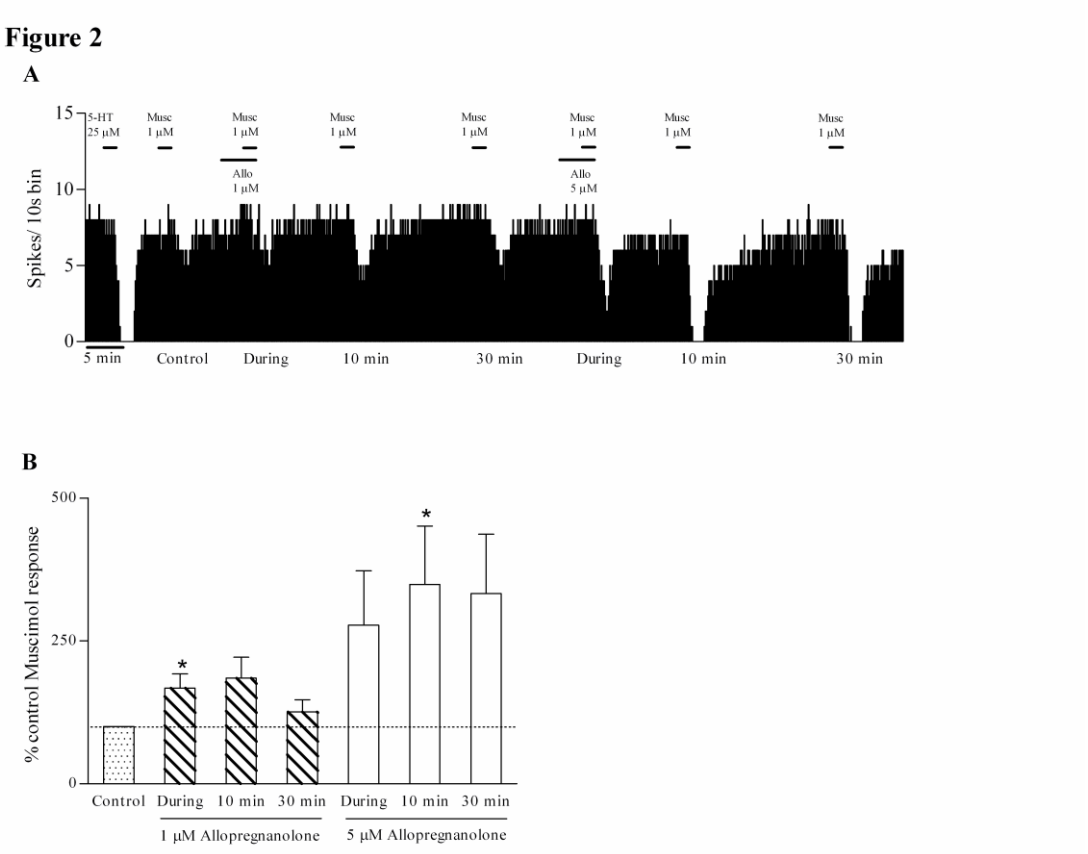

Figure 2. Enhancement of inhibitory responses to the $GABA_A$ agonist muscimol by allopregnanolone. (A) Example of a recording from an individual DRN 5-HT neuron showing responses to muscimol (Musc, 1 μM) before (control), during and after (10 min, 30 min and 60 min) the application of allopregnanolone (Allo, 1 and 5 μM) for 5 min. (B) Concentration-dependent effect of allopregnanolone (1 and 5 μM) on the potentiation of inhibitory responses to muscimol (0.3-1 μM). Data are shown as a percentage of the control response to muscimol (n = 4) (Mean ± SEM). * $P < 0.05$, paired t-test vs. response prior to steroid application (control).

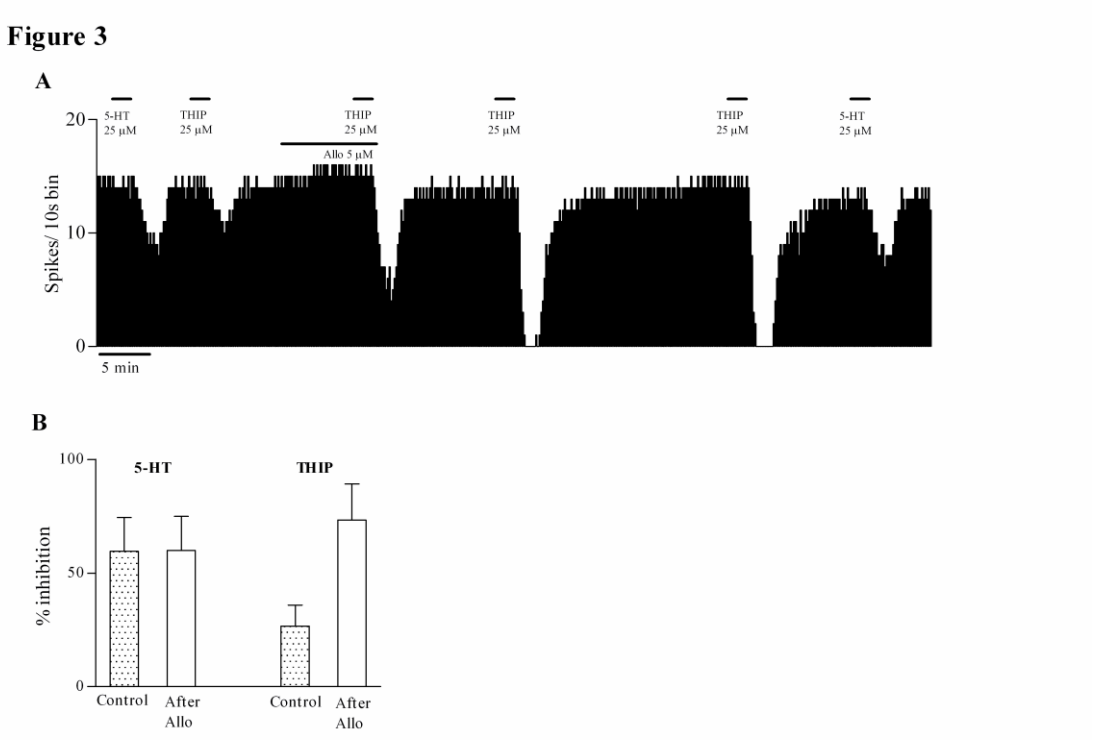

Figure 3. Allopregnanolone does not potentiate inhibitory responses to 5-HT. (A) Example of a recording showing the response of an individual DRN 5-HT neuron to 5-HT is not enhanced 45 min after allopregnanolone application. In contrast, the response to THIP is greatly potentiated at this time (approximately 45 min after application of allopregnanolone, 5 μM). (B) Bar chart showing the percentage inhibition of 5-HT neuronal firing by 5-HT (25-50 μM) and THIP (10-25 μM), before (control) and approximately 45 min after the application of allopregnanolone (n = 5). (Mean ± SEM).

Figure 4

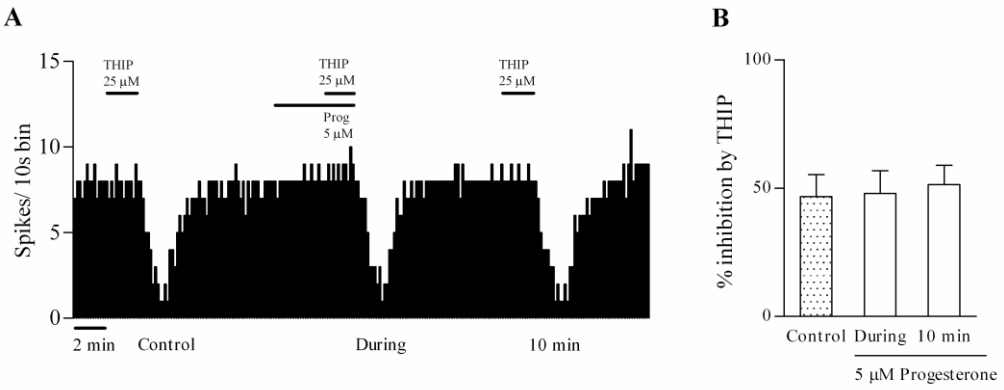

Figure 4. Progesterone has no effect on the inhibitory responses to the $GABA_A$ agonist THIP (25-50 μM). (A) Example of a recording from an individual 5-HT neuron in the DRN showing responses to THIP before (control), during and after (10 min) the application of progesterone (Prog, 5 μM) for 5 min. Basal activity was recorded in the continuous presence of the α_1-adrenoceptor agonist, phenylephrine. THIP was applied for periods of 2 min and progesterone was applied for 5 min as indicated by the bars. Note that recordings have not been corrected for the perfusion lag (approximately 2 min). (B) Mean percentage inhibition of 5-HT neuronal firing by THIP before (control), during and 10 min after 5 min application of progesterone (n = 5) (Mean ± SEM).