Serotonergic modulation of neuronal activity in rat midbrain periaqueductual gray

Hyo-Jin Jeong,* Karen Lam,* Vanessa A. Mitchell, and Christopher W. Vaughan

Pain Management Research Institute, Kolling Institute of Medical Research, Northern Clinical School, The University of Sydney at Royal North Shore Hospital, St Leonards, New South Wales, Australia

Submitted 7 September 2012; accepted in final form 14 March 2013

Jeong HJ, Lam K, Mitchell VA, Vaughan CW. Serotonergic modulation of neuronal activity in rat midbrain periaqueductual gray. J Neurophysiol 109: 2712–2719, 2013. First published March 20, 2013; doi:10.1152/jn.00790.2012.—Serotonin (5-HT) modulates pain and anxiety from within the midbrain periaqueductual gray (PAG). In the present study, the effects of 5-HT- and 5-HT1A subtype-selective ligands on rat PAG neurons were examined using whole cell patch-clamp recordings in brain slices. In voltage clamp, 5-HT produced outward and inward currents in distinct subpopulations of neurons that varied throughout different subregions of the PAG. The 5-HT1A agonist R(+)-8-OH-DPAT (1 μM) produced outward currents in subpopulations of PAG neurons. By contrast, sumatriptan (1 μM) and other 5-HT1B/D,F and 5-HT2 subtype agonists had little or no postsynaptic activity. The 5-HT2A/C agonists DOI (3 μM) and TCB-2 (1 μM) produced inward currents in subpopulations of PAG neurons, and DOI enhanced evoked inhibitory postsynaptic currents via a presynaptic mechanism. In current clamp, both R(+)-8-OH-DPAT and sumatriptan produced an excitatory increase in evoked mixed postsynaptic potentials (PSPs). In addition, R(+)-8-OH-DPAT, but not sumatriptan, directly hyperpolarized PAG neurons. By contrast, the 5-HT2 agonist DOI depolarized subpopulations of neurons and produced an inhibitory decrease in evoked mixed PSPs. These findings indicate that 5-HT1A and 5-HT1B/D,F ligands have partly overlapping inhibitory effects on membrane excitability and synaptic transmission within the PAG, which are functionally opposed by 5-HT2A/C actions in specific PAG subregions.

serotonin; PAG; postsynaptic; presynaptic; IPSP

THE NEUROTRANSMITTER SEROTONIN (5-HT) modulates a number of important physiological functions, including fear, anxiety, depression, pain, and sleep. These are mediated by a family of G protein-coupled receptors (GPCRs), including Gαq-coupled 5-HT1A/B/D,F receptors, Gq-coupled 5-HT2A/C receptors, and Gs-coupled 5-HT3, 5-HT1D, and 5-HT3 receptors as well as 5-HT3 ligand-gated ion channel (Barnes and Sharp 1999). The midbrain periaqueductal gray (PAG), like the adjacent serotonergic dorsal raphe nucleus (DRN), contains a dense plexus of serotonergic nerve terminals (Clements et al. 1985).

Many functions of the PAG overlap those of the serotonergic system. The PAG plays a pivotal role in integrating an animal’s analgesic, somatomotor, autonomic, and behavioral responses to threat, stress, and pain (Keay and Bandler 2001). These responses are organized by functionally and anatomically distinct rostrocaudally aligned columns within the PAG. Briefly, active coping strategies are organized by the ventrolateral lateral (Keay and Bandler 2001). Functional studies have shown that microinjection of 5-HT- and 5-HT1 subtype-selective agonists into the ventrolateral PAG inhibits ascending spinal and medullary nociceptive transmission, and PAG microinjection of nonselective 5-HT antagonists reduces stimulation-induced analgesia (Bartsch et al. 2004; Carstens et al. 1987; Knight and Goadsby 2001; Nichols et al. 1989). Microinjection studies have demonstrated that both 5-HT1A and 5-HT2A/C receptors within lateral/dorso-lateral PAG have a role in the modulation of anxiety (Brandao et al. 2008; Graeff 2004).

The PAG contains varying levels of 5-HT1A/B/D,F and 5-HT2A/C receptor mRNA and protein (Bonaventure et al. 1998; Bruinvels et al. 1993, 1994; Castro et al. 1997; Jeong et al. 2008; Pompeiano et al. 1992, 1994). Cellular in vitro studies have shown that activation of 5-HT1A, -B, and -D receptors presynaptically inhibits synaptic transmission within the PAG (Jeong et al. 2008; Kishimoto et al. 2001). Whereas it has been shown that postsynaptic 5-HT1A receptor activation opens an inwardly rectifying potassium conductance in acutely isolated PAG neurons (Jeong et al. 2001), postsynaptic 5-HT1A actions have not been examined in intact PAG slices, and the role of other 5-HT1 receptor subtypes and of 5-HT2 receptors has not been examined. In the present study, we examined the effect of 5-HT1 and -2 receptor subtypes on neuronal excitability throughout the different subregions of the PAG in intact PAG slices.

METHODS

Slice preparation. Experiments were carried out on male and female Sprague-Dawley rats (15–24 days old) following the guidelines of the National Health and Medical Research Council Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and with the approval of the Royal North Shore Hospital Animal Care and Ethics Committee. Animals were deeply anaesthetized with isoﬂurane and decapitated, and coronal midbrain slices (300 μm) containing PAG were cut using a vibratome (VT1000S; Leica Microsystems, Nussloch, Germany) in ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (in mM): 126 NaCl, 2.5 KCl, 1.4 NaH2PO4, 1.2 MgCl2, 2.4 CaCl2, 11 glucose, and 25 NaHCO3, as described previously (Drew et al. 2008). The slices were maintained at 34°C in a submerged chamber containing ACSF equilibrated with 95% O2 and 5% CO2. Individual slices were then transferred to a chamber and superfused continuously (1.8 ml/min) with ACSF at 34°C.

Electrophysiology. PAG and DRN (medial subdivision) neurons were visualized using Dodt-tube contrast gradient optics on an upright microscope (BX50; Olympus, Tokyo, Japan). Whole cell recordings were made using an Axopatch 200B (Molecular Devices, Sunnyvale, CA) patch clamp amplifier. Recordings were made using standard patch clamp recording protocols.
CA). In voltage-clamp experiments examining postsynaptic actions, the internal solution comprised (in mM): 95 K-gluconate, 30 KCl, 15 NaCl, 2 MgCl₂, 10 HEPES, 11 EGTA, 2 MgATP, and 0.25 NaGTP. In voltage-clamp experiments examining effects on GABAergic synaptic transmission, the internal solution comprised (in mM): 140 K- CsCl, 10 HEPES, 0.2 EGTA, 1 MgCl₂, 2 MgATP, 0.3 NaGTP, and 3 QX-314. In current-clamp experiments, the internal solution comprised (in mM): 140 K-gluconate, 10 HEPES, 11 EGTA, 2.4 MgCl₂, 2 MgATP, and 0.25 NaGTP. The internal solutions had a pH of 7.3 and osmolality of 280–285 mosmol/L, and liquid junction potentials were corrected. Series resistance (<30 MΩ) was compensated by 80% and continuously monitored during experiments.

In some experiments, electrically evoked postsynaptic currents and potentials (PSPs) were elicited in neurons via unipolar glass electrodes (containing ACSF) placed 50–150 μm lateral to the recording electrode (stimuli: 5–50 V, 20–400 μs). In voltage-clamp experiments, neurons were held at −60 mV, and GABA(A)-mediated evoked inhibitory postsynaptic currents (IPSCs) were elicited in ACSF containing the non-NMDA glutamate receptor antagonist CNQX (5 μM) and the glycine receptor antagonist strychnine (5 μM). In current-clamp experiments, the holding current was adjusted so that the initial membrane potential was 5–15 mV below action potential threshold, and PSPs were elicited in standard ACSF (as in Chiu and Huang 1999).

Analysis. Voltage-clamp and current-clamp recordings of membrane current and voltage, respectively, were filtered (2- and 10-kHz low-pass filters) and sampled (5 and 20 kHz) for later analysis (AxoGraph X; AxoGraph Scientific Software, Sydney, Australia). For voltage-clamp examination of direct postsynaptic effects, agonist-induced currents were measured from the top of the current trace. This avoided inclusion of IPSCs/excitatory postsynaptic currents (EPSCs) that were all inward with the K-gluconate/KCl-based internal recording solution [Fig. 1A, insets i and ii; IPSC and EPSC, reversal potential (E<sub>rev</sub>) = −25 and +10 mV]. For voltage-clamp examination of effects on GABAergic synaptic transmission, the amplitude of evoked IPSCs (E<sub>ev</sub> = 0 mV with CsCl-based internal solution) was measured as the peak of the IPSC relative to a 5-ms baseline period immediately before the stimulus artifact. For current-clamp recordings, the integral of evoked mixed PSPs was measured (integrated area under the PSP waveform) over a 100-ms period relative to a 5-ms baseline period immediately before the stimulus artifact (AxoGraph X). These mixed PSPs comprised both inhibitory and excitatory components (IPSPs and EPSPs, E<sub>rev</sub> = −90 and +10 mV), and thus the integral of the evoked PSP had negative and positive components for IPSPs and EPSPs, respectively. Neurons in which action potentials occurred during evoked PSPs were excluded from the analysis of evoked PSP integral. Neurons were considered to be responders if there was a change in holding current >5 pA (voltage-clamp), a change in membrane potential >3 mV (current-clamp), or a change in the amplitude/integral of evoked IPSCs/EPSPs >10%, which reversed on agonist washout or addition of an antagonist. All numerical data are expressed as means ± SE. Statistical comparisons of mean drug effects were made using Student’s paired t-test. Comparisons of proportions were made using χ² or Fisher’s exact tests. Differences were considered significant if P < 0.05.

Drug solutions. Stock solutions of all drugs were prepared in distilled water, or dimethyl sulfoxide, and then diluted (1:3,000 to 1:10,000, final vehicle concentration 0.01–0.033% vol/vol) to working concentrations using ACSF and applied by superfusion. Supramaximal concentrations of agonists and antagonists were used (as in Jeong et al. 2008). 1,4-Dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5H-pyrrol[3,2-b]pyridin-5-one dihydrochloride (CP 93129), 2-[5-[3-(4-methylsulfonylamino)benzyl]-1,2,4-oxadiazol-5-yl]-1H-indol-3-yl]ethanamine (L-694,247), N-((3R)-3-(dimethylamino)-2,3,4,9-tetrahydro-1H-carbazol-6-yl)-4-fluorobenzamide hydrochloride (LY344864), (S)-3,4-dihydro-1-[2-[4-(4-methoxyx-p-henyl)-1-piperazinyl]ethyl]N-methyl-1H-2-benzopyr-an-6-carboxamide (PNU 109291), (2R)-(+)-8-hydroxy-2-(di-N-propylamino)tetralin hydrobromide [(R+)-8-OH-DPAT], (4-bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylymide hydrobromide (TCB-2), and (S)-N-tert-buty1-3-(4-(2-methoxyphenyl)piperazin-1-yl)-2-phenylpropamide dihydrochloride (WAY 101135) were obtained from Tocris Cookson (Bristol, United Kingdom); 6-cyano-7-nitroquinolinoxaline-2,3-dione (CNQX), dl-(−)-2-amino-5-phosphonopentanoic acid (AP5), QX-314 bromide, and TTX were from Ascent Scientific (Bristol, United Kingdom); (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminoamphetamine hydrochloride, (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride [(±)-DOI], 5-HT, methionine enkephalin, 3-[2-(dimethylamino)ethyl]N-methyl-1H-indole-5-methanesulfonyamide succinate (sumatriptan), N-[2-[4-(2-methoxyphenyl)-1-piperaziny1]ethyl]-N-2-pyridinylcyanohexancarbamide maleate (WAY 106635), and all other reagents were from Sigma-Aldrich (Sydney, Australia).

RESULTS

Postsynaptic actions of 5-HT in PAG. When neurons were voltage-clamped at −60 mV, administration of a maximal concentration of 5-HT (10 μM) onto midbrain slices produced...
a range of postsynaptic current responses (Fig. 1, A–C). 5-HT produced an outward current in 48% of PAG neurons that reversed following washout (Fig. 1A; mean current = 30 ± 3 pA, n = 40). 5-HT produced an inward current in 20% of PAG neurons that slowly reversed following washout (Fig. 1C; mean current = −16 ± 3 pA, n = 16). In the other 33% of PAG neurons, 5-HT produced no change in membrane current (Fig. 1B; mean current = 0 ± 1 pA, n = 28). Subsequent application of the GABAB agonist baclofen (10 μM) produced an outward current that was not significantly different among the three types of 5-HT-responding neurons (Fig. 1, A–C; P = 0.2; mean current = 37 ± 3 pA, n = 84).

We examined whether these responses differed throughout subregions of the PAG and compared this with the adjacent DRN (Calizo et al. 2011; Marinelli et al. 2004). The relative proportions of 5-HT-responding types differed among neurons in the ventrolateral, lateral, and dorsolateral columns and the DRN (Fig. 1D; P = 0.01, χ² = 16.1, degrees of freedom (df) = 6). This difference was largely due to a lower proportion of neurons that responded to 5-HT with an inward current in the ventrolateral PAG compared with the lateral and dorsolateral PAG and the DRN (Fig. 1D; P = 0.036, χ² = 8.55, df = 3). The endogenous opioid met-enkephalin (10 μM) produced an outward current in similar proportions of the three types of 5-HT-responding neurons (P = 0.7, χ² = 0.6, df = 2). Met-enkephalin (10 μM) produced an outward current in 68% (n = 13/19) and 80% (n = 8/10) of neurons that responded to 5-HT with outward and inward currents, respectively, and in 67% (n = 12/18) of neurons that did not respond to 5-HT. These findings indicate that 5-HT produces inward and outward currents in different subpopulations of PAG neurons that vary throughout the different columns but are independent of their opioid responsiveness.

**Actions of 5-HT₁ subtype-selective agonists.** We next examined the effect of a range of 5-HT₁ subtype-selective agonists on membrane current in PAG neurons. The 5-HT₁₄ agonist R(+)–8-OH-DPAT (1 μM) produced an outward current in 53% of PAG neurons tested (Fig. 2, A and F; n = 18/34). The 5-HT₁₃ agonist CP 93129 (1 μM) did not produce a change in membrane current in most neurons (Fig. 2B), producing an outward current in only 18% of PAG neurons (Figs. 2A and 2F; n = 4/22). Whereas the 5-HT₁₅ agonist L-694,247 (1 μM) produced an outward current in 30% of PAG neurons (Figs. 2F and 3B; n = 3/10), another 5-HT₁₃ agonist PNU 109291 (1 μM) did not produce a change in membrane current in any PAG neurons (Fig. 2, C and F; n = 8). The 5-HT₁₇ agonist LY344864 (1 μM) did not produce a change in membrane current in any PAG neurons (Fig. 2, B and F; n = 12). The
antimigraine drug sumatriptan (1 μM) did not produce a change in membrane current in any PAG neurons (Fig. 2, D and F; n = 8). A higher concentration of sumatriptan (10 μM) produced an outward current in 6% of neurons (Fig. 2E; n = 1/18). It can be noted that R(+)-8-OH-DPAT, CP 93129, and sumatriptan (1 μM) also produced a reduction in spontaneous synaptic currents even in cases where they did not produce a change in holding current (Fig. 2, A, B, and D).

In responding neurons, the outward current produced by R(+)-8-OH-DPAT was reversed by addition of either of the 5-HT1A antagonists WAY 100135 (1 μM) and CP 93129, and sumatriptan (1 μM) produced an inward current in 43% of PAG neurons (Fig. 2F; n = 3/7). The 5-HT2A agonist TCB-2 (1 μM) produced an inward current in 17% of PAG neurons tested (Fig. 2, E and F; n = 2/12). Of the neurons tested with DOI and TCB-2, 50% (n = 2/4), 38% (n = 3/8), and 0% (n = 0/7) were in the dorsolateral, lateral, and ventrolateral PAG.

We have previously demonstrated that 5-HT inhibits GABAergic synaptic transmission within PAG and that this is mimicked by 5-HT1A receptor agonists as well as sumatriptan and 5-HT1B/D receptor agonists (Jeong et al. 2008). Given the above finding that 5-HT2A/C agonists have postsynaptic effects on PAG neurons, we also examined whether DOI modulates GABAergic synaptic transmission. In these experiments, we focused on the lateral and dorsolateral columns where 5-HT2 postsynaptic currents were observed. In these neurons, paired evoked IPSCs were elicited by two stimuli of identical strength in close succession (evoked IPSC2:1; interstimulus interval = 70 ms) in the presence of CNQX (5 μM) and strychnine (3 μM). Superfusion of DOI (3 μM) produced an increase in the amplitude of evoked IPSCs in subpopulations of lateral (n = 3/5) and dorsolateral (n = 1/4) PAG neurons that was significant when averaged across all neurons tested (Fig. 4, A and B; P = 0.03; n = 9). In these neurons, there was a decrease in the ratio of evoked IPSC2:1 (92 ± 3%, n = 9; P = 0.02; Fig. 4, A and B). These findings indicate that 5-HT2 ligands produce inward currents and enhance GABAergic synaptic transmission in subpopulations of lateral and dorsolateral PAG neurons.

Sero
tonergic effects on net neuronal excitability. In conjunction with prior studies, the above experiments suggest that the 5-HT1/2 receptor subtypes have distinct actions at pre- and postsynaptic sites. We next examined how these actions combined to alter net neuronal excitability by examining the effect of the 5-HT12 subtype-selective agonists R(+)-8-OH-DPAT, sumatriptan, and DOI on basal and evoked neuronal excitability in current-clamp mode. In these experiments, a K-gluconate-based internal solution was used to examine the effect on membrane potential and evoked mixed PSPs.

Fig. 3. Outward currents produced by 5-HT1B/D ligands are mediated by 5-HT1A receptors. A and B: current traces of 2 different neurons during superfusion of the 5-HT1B and -D agonists CP 93129 (1 μM) and L-694,247 (1 μM) and addition of the 5-HT1A-B, and -D antagonists WAY 100135 (3 μM), NAS181 (1 μM), and BRL15572 (BRL; 1 μM). C: bar chart of the mean current produced by 8-OH-DPAT (1 μM) and CP 93129 (1 μM) and the effect on these currents of addition of 5-HT1B antagonist NAS181 (3 μM) and/or 5-HT1A antagonists WAY 100135 and WAY 100635 (1 μM). In C, * and ** denote P < 0.05, 0.01. Neurons were voltage-clamped at −60 mV.
The 5-HT\textsubscript{1A} agonists had actions in current-clamp that were consistent with those observed in voltage clamp. R(+)-8-OH-DPAT (1 \text{ \& M}) hyperpolarized 83\% of neurons tested (n = 10/12), and this was significant when averaged across all neurons (Fig. 5, A and D; P = 0.0004). Sumatriptan (1 \text{ \& M}) had no effect on membrane voltage in any neuron tested (Fig. 5, B and D; P = 0.9; n = 7). By contrast, DOI (3–10 \text{ \& M}) depolarized 33\% of neurons tested (Fig. 5, C and D; n = 3/9; P = 0.03), and in one neuron, this resulted in the generation of action potentials (Fig. 5C).

In these neurons, electrical stimulation evoked mixed PSPs that usually comprised overlapping IPSPs and EPSPs (Fig. 5, A–C, and Fig. 6, A–C). These IPSCs and EPSCs had varying kinetics and were blocked by picrotoxin (100 \text{ \& M}) and CNQX (10 \text{ \& M}), respectively. In most neurons, application of R(+)-8-OH-DPAT and sumatriptan produced an excitatory increase in the integral of the evoked mixed PSP that was significant when averaged across all neurons (Fig. 5E; P = 0.001, 0.01; n = 12, 6). The increase in the integral of the evoked mixed PSP was greater for sumatriptan than for R(+)-8-OH-DPAT (Fig. 5E; P = 0.03). This was usually observed as a reduction in the evoked IPSP and the unmasking of an evoked EPSP (Fig. 5, A and B). It was possible that the increase in evoked mixed PSPs produced by R(+)-8-OH-DPAT was due to change in PSP driving force arising from the hyperpolarization. In five of the above neurons, R(+)-8-OH-DPAT produced an increase in the mixed PSP integral of 167 ± 17 \text{ \text{mV}} \cdot \text{ms} when hyperpolarized (P = 0.0005). When the neurons were repolarized to the pre-R(+)-8-OH-DPAT value, the increase mixed PSP integral was 103 ± 17 \text{ \text{mV}} \cdot \text{ms}, which was less than that in the hyperpolarized state (P = 0.03) but still significant (P = 0.004). Thus the effect of R(+)-8-OH-DPAT on PSPs was not solely a consequence of membrane hyperpolarization. In contrast to the 5-HT\textsubscript{1A} ligands, DOI usually produced an inhibitory decrease in the integral of evoked mixed PSPs in most neurons, which was observed as an increase in evoked IPSPs (Fig. 5, C and E; n = 6/9; P = 0.04).

Given that 5-HT\textsubscript{1A}, 5-HT\textsubscript{1B/D}, and 5-HT\textsubscript{2AC} agonists have differential pre- and postsynaptic effects, we then examined the net action of 5-HT on neuronal excitability. Application of 5-HT (10 \text{ \& M}) had a variable effect in current-clamp mode, hyperpolarizing 50\% of neurons and depolarizing 25\% of neurons tested (Fig. 6, A–C and D; n = 8, 4). 5-HT had no effect on the resting membrane voltage in the other neurons (Fig. 6D; n = 4). In these neurons, 5-HT also had a variable effect on evoked mixed PSPs. The 5-HT-induced hyperpolarization was usually associated with an increase in the integral of evoked mixed PSPs (Fig. 6, A, B, and D; n = 4/9). The increase in the evoked mixed PSP was usually observed as a reduction in IPSPs and the unmasking of EPSPs (Fig. 6A). Despite the membrane hyperpolarization, this led to an increase in transient action potential firing during the evoked PSP in two neurons (Fig. 6B). By contrast, the 5-HT-induced depolarization was always associated with an inhibitory decrease in the integral of evoked mixed PSPs and was observed as an increase in IPSPs (Fig. 6, C and D; n = 3/3).

DISCUSSION

The present findings suggest that 5-HT\textsubscript{1A}, 5-HT\textsubscript{1B/D}, and 5-HT\textsubscript{2AC} receptor activation modulates neuronal excitability

![Figure 4](http://jn.physiology.org/)

![Figure 5](http://jn.physiology.org/)
within the PAG via partly overlapping pre- and postsynaptic mechanisms. 5-HT_{1A} and 5-HT_{2A/C} receptors regulated PAG activity at the postsynaptic level, whereas 5-HT_{1B/D} and 5-HT_{2A/C} receptors modulated presynaptic inputs onto PAG neurons. In addition, the 5-HT_{1A} and 5-HT_{2} receptor subtypes exerted functionally opposite actions at both pre- and postsynaptic levels.

In the present study, it was found that 5-HT produced either an outward inhibitory current (46%) or an inward excitatory current (18%) or had no postsynaptic effect (36%) in PAG neurons under voltage-clamp conditions. These proportions differ from that previously observed in acutely dissociated PAG neurons where 5-HT produced an outward inhibitory current in 35% of neurons and no inward excitatory currents were observed (Jeong et al. 2001). This difference may be due to the use of slices vs. acutely isolated neurons. It was also found that the proportions of 5-HT-responding types varied throughout the PAG. A greater proportion of neurons responded to 5-HT with an inward current in the lateral and dorsolateral PAG (26%) compared with the ventrolateral PAG (4%). It can be noted that 33% of neurons in the DRN, which is adjacent to the ventrolateral PAG, responded to 5-HT with an inward excitatory current as observed previously (Beck et al. 2004; Craven et al. 2001; Kirby et al. 2003; Liu et al. 2000; Marinelli et al. 2004; Xu et al. 1998). These observations indicate that there is a high degree of organization of postsynaptic 5-HT actions within the PAG.

The 5-HT-induced outward and inward currents were likely to be mediated by 5-HT_{1A} and 5-HT_{2A/C} receptors, respectively. The 5-HT_{1A}-selective agonist R(+)-8-OH-DPAT produced an outward current under voltage-clamp conditions that was reversed by addition of the 5-HT_{1A} antagonists WAY 100135 and WAY 100635. This inhibitory action is similar to the activation of a postsynaptic inwardly rectifying K\(^+\) conductance by the less active isomer 8-OH-DPAT observed previously in PAG neurons (Jeong et al. 2001). The 5-HT_{1B} agonist CP 93129 and the 5-HT_{1D} agonist L-694,247 produced an outward current in only a small proportion of PAG neurons, whereas another 5-HT_{1D} agonist, PNU 109291, and the 5-HT_{1F} agonist LY344864 had no effect in any PAG neurons. In addition, the antimigraine drug sumatriptan, which is an agonist at 5-HT_{1B/D} and B receptors, had no effect at a 1 \(\mu\)M concentration and produced an outward current in only 1/18 neurons at a 10 \(\mu\)M concentration. The CP 93129- and L-694,247-induced currents were likely to be 5-HT_{1A} receptor-mediated because they were unaffected by 5-HT_{1B} and 5-HT_{1D} antagonists and were reversed by 5-HT_{1A} antagonists. Like prior in vitro electrophysiological studies, the 5-HT_{1} subtype agonists were used at concentrations in the low micromolar range. Although these 5-HT_{1} subtype-selective agonists have low nanomolar affinities for their respective receptors, they have variable selectivity over other 5-HT_{1} receptor subtypes. For example, PNU 109291 has >1,000-fold selectivity for 5-HT_{1D} vs. 5-HT_{1A} and 5-HT_{1B} receptors, but sumatriptan (5-HT_{1B/D} vs. 5-HT_{1A}), CP 93129 (5-HT_{1B} vs. 5-HT_{1A} and 5-HT_{1D}), and L-694,247 (5-HT_{1D} vs. 5-HT_{1A} and 5-HT_{1D}) have only an 8- to 200-fold selectivity for their respective targets (Beer et al. 1993; Ennis et al. 1998; Macor et al. 1990). This difference in selectivity was consistent with that observed in our study and indicates that the actions of 5-HT_{1B/D} agonists should be confirmed with antagonists because they can produce 5-HT_{1A} receptor-mediated effects at low micromolar concentrations. The 5-HT_{2A} and 5-HT_{2A/C} agonists TCB-2 and DOI both produced an inward excitatory current in subpopulations of PAG neurons, which is similar to that observed by others in the adjacent DRN (Calizo et al. 2011; Craven et al. 2001; Liu et al. 2000; Marinelli et al. 2004).

In the present study, it was also shown that 5-HT_{2A/C} receptor activation enhanced GABA\(_{A}\)-mediated synaptic transmission in a subpopulation of PAG neurons. This was likely to be mediated by a presynaptic mechanism because the increase in evoked IPSCs was associated with a change in their paired-pulse ratio. This differs from and would functionally oppose the 5-HT_{1A}- and 5-HT_{1B/D}-induced presynaptic inhibition that we and others have observed previously within the PAG and DRN (Jeong et al. 2008; Kishimoto et al. 2001; Lemos et al. 2006). It can be noted that this 5-HT_{2A/C}-mediated presynaptic enhancement was observed in the lateral and dorsolateral PAG,
the columns in which the 5-HT$_{2AC}$-induced postsynaptic depolarization was most prominent. The actions of 5-HT$_2$ in the PAG therefore differ from the adjacent DRN where enhanced GABAergic synaptic transmission is solely due to postsynaptic depolarization and action potential-induced increases in IPSC frequency (Liu et al. 2000).

In conjunction with prior studies, the present voltage-clamp experiments indicate that different 5-HT receptors subtypes have distinct pre- and postsynaptic actions. Under current-clamp conditions, these distinct mechanisms combined to alter net neuronal excitability. The 5-HT$_{1A}$ and 5-HT$_{1B/D}$ agonists R(+)-8-OH-DPAT and sumatriptan both produced an excitatory increase in evoked mixed PSPs in subpopulations of neurons that in some cases resulted in transient action potential firing during the evoked PSP. The 5-HT$_{1A}$ and 5-HT$_{1B/D}$ agonist-induced excitatory increase in evoked mixed PSPs was likely to be presynaptically mediated because it was observed in the absence of postsynaptic changes in membrane voltage. These evoked mixed PSPs were a combination of GABAergic IPSPs and glutamatergic EPSPs (see also Chiu and Huang 1999). Thus the excitatory increase in the evoked mixed PSPs was likely to be due a reduction in GABAergic evoked IPSPs, which is mediated by presynaptic 5-HT$_{1A}$ and 5-HT$_{1B/D}$ receptor inhibition of GABA release (Jeong et al. 2008; Kishimoto et al. 2001). It can be noted, however, that 5-HT$_{1A}$ and 5-HT$_{1B/D}$ activation also presynaptically inhibits glutamate release within PAG (Jeong et al. 2008). This suggests that both 5-HT$_{1A}$ and 5-HT$_{1B/D}$ activation produce greater presynaptic inhibition of IPSPs compared with EPSPs, as observed previously for opioids within PAG (Chiu and Huang 1999). There were, however, subtle differences between the effects of R(+)-8-OH-DPAT and sumatripan in current-clamp mode. First, the excitatory increase in evoked PSPs produced by sumatripan was greater than that for R(+)-8-OH-DPAT. This is consistent with the relatively greater presynaptic inhibition produced by 5-HT$_{1B/D}$ receptors compared with 5-HT$_{1A}$ receptors (Jeong et al. 2008). Second, R(+)-8-OH-DPAT but not sumatripan directly hyperpolarized subpopulations of neurons. This is consistent with our finding that 5-HT$_{1A}$ but not 5-HT$_{1B/D}$ activation produced an outward current in voltage clamp. Overall, these findings indicate that although both 5-HT$_{1A}$ and 5-HT$_{1B/D}$ activation presynaptically enhances evoked neuronal activity, 5-HT$_{1A}$ activation also reduces background activity via postsynaptic inhibition.

In contrast to 5-HT$_1$ agonists, the 5-HT$_{2AC}$ agonist DOI depolarized a subpopulation of PAG neurons, which is consistent with the inward current induced in voltage clamp. In addition, DOI produced an inhibitory decrease in electrically evoked mixed PSPs in subpopulations of neurons. This was likely to be due to a presynaptic enhancement of the evoked IPSPs because DOI and TCB-2 both enhanced evoked IPSCs under voltage-clamp conditions. These findings indicate that 5-HT$_{2AC}$ activation presynaptically inhibits evoked neuronal activity but enhances background activity via postsynaptic excitation. This would functionally oppose the 5-HT$_{1A}$ and 5-HT$_{1B/D}$ receptor-mediated actions at both pre- and postsynaptic levels. Indeed, it was found that 5-HT had complex actions on neuronal activity that comprised distinct actions via pre- and postsynaptic 5-HT$_{1A}$ and 5-HT$_{2AC}$ receptors and via presynaptic 5-HT$_{1B/D}$ receptors.

Like opioids and cannabinoids, serotonergic systems within the PAG modulate pain and anxiety. In combination with prior studies, we have found that 5-HT$_{1A/B/D}$ and 5-HT$_{2AC}$ receptor systems exert opposing postsynaptic actions and presynaptic modulation of GABAergic synaptic transmission in subpopulations of PAG neurons. These actions are more complex than those of opioids that directly inhibit a subpopulation of PAG neurons and both opioids and cannabinoids that presynaptically inhibit GABAergic and glutamatergic synaptic transmission throughout the lateral/ventrolateral PAG (Chieng and Christie 1994; Chiu and Huang 1999; Vaughan and Christie 1997; Vaughan et al. 2000). The present study suggests that 5-HT$_{1A}$ and 5-HT$_{2AC}$ receptors have functionally opposing pre- and postsynaptic actions within the lateral/dorsolateral PAG, which is consistent with their contrasting actions on anxiety within these PAG columns (de Paula Soares and Zangrossi 2009; Graeff et al. 1993; Nogueira and Graeff 1991; Zanoveli et al. 2003). The presynaptic modulation of neuronal excitability by 5-HT$_{1B/D}$ receptors within these columns adds another component to anxiolytic control within this brain structure. Endogenous analgesia within the PAG is mediated via distinct non-opioid and opioid systems within the lateral/dorsolateral and ventrolateral PAG, respectively (see Hohmann et al. 2005). Although microinjection of 5-HT into the PAG has an analgic action, the PAG columns and 5-HT receptor subtypes involved remain to be determined (Carstens et al. 1987; Nichols et al. 1989), although 5-HT$_{1B/D}$ receptors in the ventrolateral PAG have been implicated in analgesia specific to pain of intracranial origin (Bartsch et al. 2004; Knight and Goadsby 2001). The present study found that the ventrolateral PAG differs from the other PAG columns in that there is reduced 5-HT$_{2AC}$ activity within this column. Thus, although functionally opposing 5-HT$_{1A/B/D}$ and 5-HT$_{2AC}$ actions could potentially modulate analgesia within the lateral PAG, 5-HT$_{1A/B/D}$ actions will predominate in the ventrolateral PAG. Overall, the present study indicates 5-HT will have wide ranging roles in the modulation of pain and anxiety within this brain region and that subtype-selective ligands might be used to target specific functions within this brain structure.

GRANTS

This work was supported by National Health and Medical Research Council of Australia Grant 632546 (C. W. Vaughan and H.-J. Jeong).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


