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Elicited PGO Waves in Rats: Lack of 5-HT_{1A} Inhibition in Putative Pontine Generator Region

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SANFORD, L. D., S. M. TEJANI-BUTT, R. J. ROSS AND A. R. MORRISON. *Elicited PGO waves in rats: Lack of 5-HT_{1A} inhibition in putative pontine generator region.* PHARMACOL BIOCHEM BEHAV **53**(2) 323–327, 1996.—Ponto-geniculo-occipital (PGO) waves and an elicited analogue (PGO_E) may be recorded in the pons of rats. Cholinergic cells in the pedunculopontine tegmental (PPT) and laterodorsal tegmental (LDT) nuclei are implicated in the generation of PGO waves. Serotonin (5-HT) may inhibit the generation of PGO waves, and possibly PGO_E. We examined the role of 5-HT_{1A} receptor mechanisms in the generation of auditory-elicited PGO_E in rats. Administration of 8-OH-DPAT [8-hydroxy-2-(*n*-dipropylamino)tetralin] into PPT did not significantly affect PGO_E amplitude or response frequency. Binding of [^{3}H]CN-IMI ([^{3}H]cyanoimipramine) to 5-HT uptake sites located presynaptically was used as a measure of 5-HT innervation. Quantitative autoradiographic analysis of [^{3}H]CN-IMI binding indicated a moderate to low degree of 5-HT innervation of PPT and a moderately high innervation of LDT compared to the dorsal raphe nucleus (DRN). Binding of [^{3}H]8-OH-DPAT to 5-HT_{1A} receptors revealed few receptor sites in PPT, and a low to moderate number of receptors in LDT compared to binding in DRN. The results suggest that inhibitory serotonergic modulation of PGO_E is probably not mediated through a 5-HT_{1A} receptor mechanism in PPT.

8-OH-DPAT Elicited PGO waves Laterodorsal tegmental nucleus Pedunculopontine tegmental nucleus PGO waves Serotonin

PONTO-GENICULO-OCCIPITAL (PGO) waves are macropotential waveforms that can be recorded in widespread regions of the brain, most notably the pons, the lateral geniculate bodies (LGB), and the occipital cortex, from which their name is derived. In rats, PGO waves may be recorded in the pons in the vicinity of the locus coeruleus (LC) and in the gray matter of the cerebellum (6,10,15,16,21,22), although they have not been recorded in LGB. Elicited waves (PGO_E) may be recorded in cats (1) and rats (16) from the same neural structures that yield spontaneously occurring PGO waves. In cats, the generation of PGO waves, and possibly PGO_E, has been traced to putative cholinergic neurons in the pedunculopontine (PPT) and lateral dorsal tegmental (LDT) nuclei that fire in bursts 15–20 ms prior to a PGO wave in LGB (23,26,30). Recently, neurons with firing characteristics fitting the criteria for PGO wave burst neurons have been described in slice preparations of LDT taken from rat pups (20), although such neurons were not found in vivo in adult rats (17).

Research suggests that 5-HT, probably originating in the dorsal raphe nucleus (DRN), inhibits the generation of PGO waves. In cats, pharmacological depletion of 5-HT (2,5,14), lesions of the DRN, and cuts between the DRN and the dorsolateral pons release PGO waves into states other than REM sleep (REM) (35), whereas electrical stimulation of DRN sup-

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presses PGO wave activity during REM (13). In rats, cholinergic neurons in PPT/LDT are inhibited in vitro by 5-HT (19,20). Cholinergic "burst" neurons in LDT are hyperpolarized by 5-HT and the 5-HT_1 agonist 5-CT (carboxamidotryptamine maleate) (20).

We examined whether local administration into PPT of the relatively specific 5-HT_{1A} agonist, 8-OH-DPAT, would suppress PGO_E during waking in rats. We focused on PGO_E because the eliciting stimulus could be presented at specific intervals after the drug was given; thus, we could reliably produce PGO_E and assess the drug's effect at the equivalent time during the drug's course of action. We chose to microinject into PPT, and not LDT, because work in cats has, thus far, more firmly established a link between PGO wave generation and PPT, and lesions of LDT do not abolish PGO waves in cats (34).

To determine whether PPT and/or LDT in rats receive serotonergic innervation consistent with the hypothesized role for 5-HT in inhibiting PGO wave generation, we measured, in a separate group of rats, the binding of [^{3}H]CN-IMI ([^{3}H]cyanoimipramine) to 5-HT uptake sites located presynaptically as a measure of 5-HT innervation. Binding of [^{3}H]CN-IMI in PPT and LDT was compared to that in DRN, a 5-HT cell body area (18). To determine whether the putative inhibitory serotonergic modulation of PGO waves in rats might involve 5-HT_{1A} receptors, we measured the binding of [^{3}H]8-OH-DPAT to 5-HT_{1A} receptors in PPT and LDT (11).

METHOD

Subjects

The subjects were male, Sprague–Dawley rats between 90 and 120 days old at the time of surgery. The rats were maintained on a 12 L : 12 D cycle and given ad lib food and water for the duration of the experiment.

Surgery

The rats were anesthetized with IP injections of ketamine (85 mg/kg) and xylazine (12 mg/kg). All surgery was performed under aseptic conditions. The rats were implanted with screw electrodes for recording EEG. Bipolar electrodes were stereotaxically implanted in the vicinity of LC [coordinates AP −0.3 (intra-aural zero), ML 1.0, DV 7.0; Paxinos and Watson (27)] for recording PGO_E (20). Guide cannulae (26 ga., Plastic Products) were permanently affixed with their tips 1 mm above the injection site in PPT for local microinjections [coordinates AP 1.2 (intra-aural zero), ML 2.0, DV 6.0; Paxinos and Watson (27)]. The rats received the antibiotic gentamicin (6 mg, IM), and buprenorphine (0.5 mg/kg, SC) to control potential postoperative pain. A minimum of 10 postoperative recovery days elapsed prior to the animals being used in any experiments.

Procedure

For microinjections a 33-ga cannula (Plastic Products) was inserted into the guide cannula. 8-OH-DPAT (0.01, 0.1, 1.0 μg/0.2 μl; $N = 6$) was microinjected into PPT at a rate of 0.1 μl/min using a 1-μl Hamilton syringe. Drug microinjections were given in a counterbalanced order. Equal volume microinjections of saline (sal1, sal2) preceded and followed the three drug injections. Drug injections were unilateral, and recordings of PGO_E were ipsilateral to the injection site. Evidence from anatomical (36), lesion (30), and pharmacological studies (4) suggests that the greatest influence of PPT on PGO waves is ipsilateral.

After receiving an injection, the rats were placed in the recording chamber and attached to a lightweight, shielded cable for recording. The rats were allowed to habituate to the chamber prior to beginning experimental trials. The habituation period usually lasted 15–20 min for each experimental trial. Six blocks of 40 tones (90 ms, 110 dB, 4 kHz, 2 s ISI) were presented. Blocks of stimuli were separated by 15 min. Experimental sessions were separated by a minimum of 3 days.

White noise was generated by a General Radio Company random noise generator (Model #1382). Generator output was gated to a Harmon/Kardon (Model #PM655 Vxi) amplifier through Coulbourn Instruments modular components that controlled all stimulus parameters except intensity. Stimuli were presented to the rats with a JBL (Model #2105H) loudspeaker.

Autoradiographic Studies

Preparation of tissue sections. A group of control rats (300 g) was decapitated and their brains were frozen on powdered dry ice and stored at −70°C until sectioned. Coronal sections (20 μm) were cut on a cryostat at −15°C, at the level of plates 49 and 52 (27), thaw-mounted onto gelatin-coated slides (two sections/slide), and desiccated overnight at 4°C. These slide-mounted sections were then stored at −70°C until assayed.

[^{3}H]CN-IMI binding to uptake sites for 5-HT. The binding of [^{3}H]CN-IMI to uptake sites for 5-HT was measured by autoradiography using the method of Kovachich et al. (18). Sections were incubated with 0.3 nM [^{3}H]CN-IMI (New England Nuclear) in Tris buffer (50 mM Tris, 150 mM NaCl), pH 7.4, for 24 h at 4°C. Nonspecific binding, defined using 5 μM sertraline, was less than 10% of total binding. Following incubation, sections were washed in cold buffer for 60 min, dipped into cold water, and dried. Sections were loaded into cassettes for 2–3 weeks depending on the regions of the brain.

[^{3}H]-8-OH-DPAT binding to 5-HT_{1A} receptor sites. The procedure for the binding of [^{3}H]8 O-DPAT to 5-HT_{1A} receptors by autoradiography was adapted from Hensler et al. (11). Brain sections were preincubated for 30 min in Tris buffer (170 mM), pH 7.6, followed by a 60-min incubation in the same buffer containing 1 nM [^{3}H]8-OH-DPAT (New England Nuclear, specific gravity: 183–227 Ci/mM). Nonspecific binding was defined using 2 μm 5-HT and ranged from 10% to 20% of total binding, depending on the brain regions examined. Following incubation, the sections were washed at 4°C in the same buffer (2 × 5 min), dipped in cold water, and dried. Dried, slide-mounted sections were then placed into X-ray cassettes and apposed to [^{3}H]-Ultrafilm for 2–3 weeks, depending on the brain region and the specific activity of [^{3}H]8-OH-DPAT.

Quantitation of autoradiograms. All films were developed using Kodak GBX developer (3 min), dipped in water, and fixed in Kodak GBX fixer (6 min) at room temperature. The autoradiograms were analyzed on a DUMAS (Drexel Unix-based Microcomputer Imager Analysis System) densitometer using the Brain software package (7). Plastic-embedded tritium standards (American Radiolabelled Chemicals, St Louis, MO) calibrated using brain-mash sections were used for quantitation (8,9).

Data Analyses

PGO_E were detected with a custom program running a Metrabyte Das-8 A/D board installed in an IBM AT-compatible

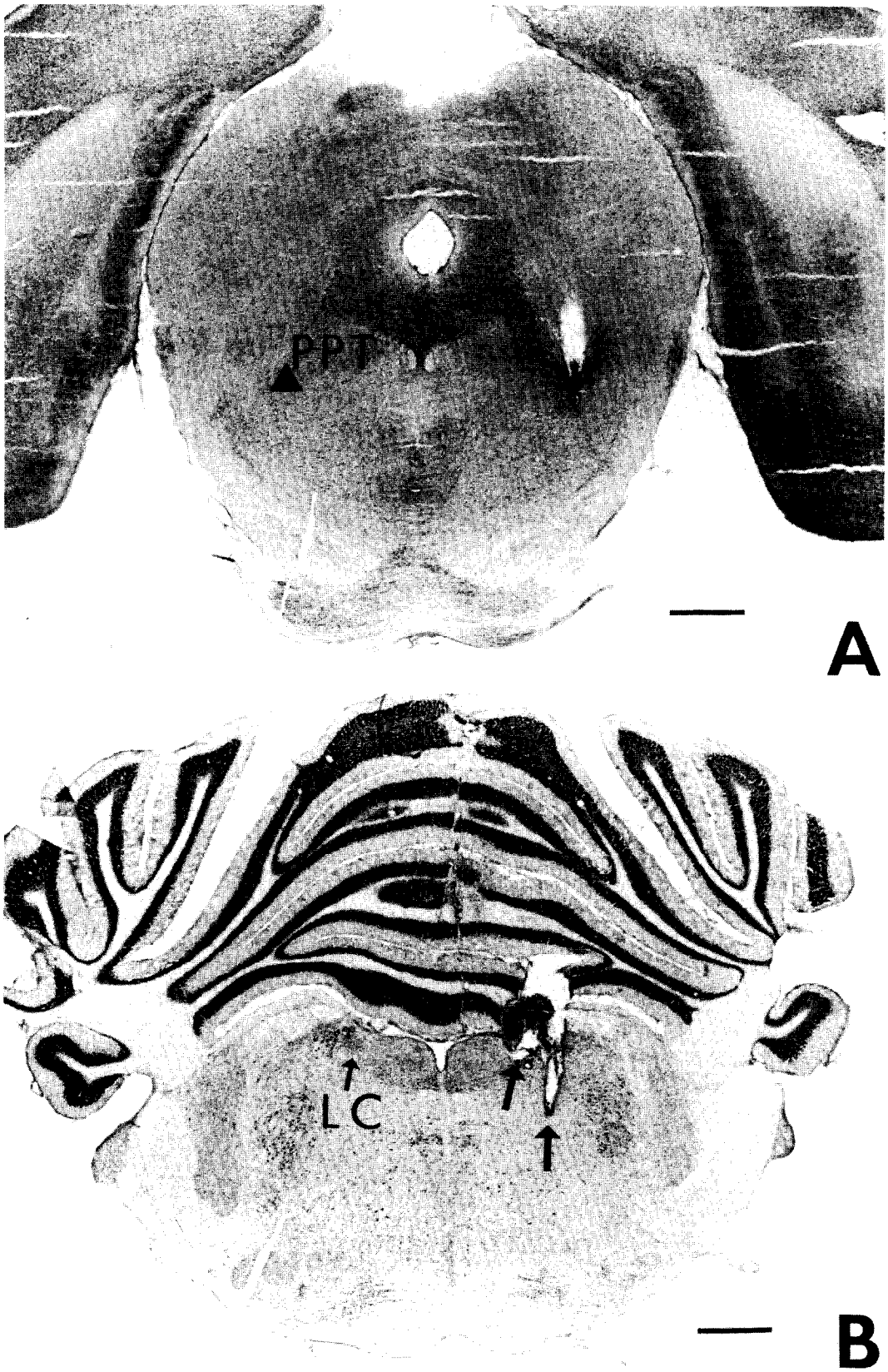

FIG. 1. Photomicrographs of coronal sections showing cannula (A) and recording electrode (B) placements in one animal. LC: locus coeruleus, PPT: pedunculopontine tegmental nucleus. The arrows in (B) point to the placements of the two poles of the bilateral recording electrode. Horizontal bar = 1.0 mm.

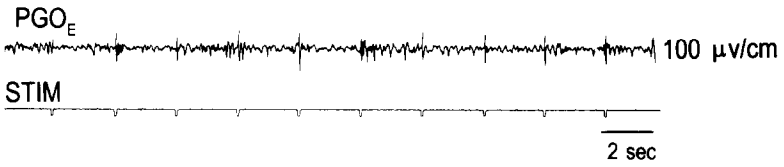

FIG. 2. Continuous polygraph record of a rat exhibiting PGO_E in wakefulness.

computer. The mean amplitude (peak height of PGO_E), mean latency (time from stimulus onset to peak height), and mean proportion (trials with PGO_E/total number of trials) were determined for each block. The amplitude, latency, and proportion data were analyzed with 5 (drug condition) × 6 (trial block) within-subjects ANOVAs.

Histological Procedures for Rats Studied in Behavioral Experiments

The rats used in the behavioral experiments were overdosed with sodium pentobarbital (50–100 mg/100 g, IP) and perfused intracardially with 9% saline and 10% formalin. The brains were processed to determine cannula and electrode placements. For this purpose, 40-μm slices were made through the areas of interest, and the sections were stained with cresyl violet. Representative electrode and cannula placements are presented in Fig. 1.

RESULTS

Local Administration of 8-OH-DPAT into PPT

Auditory stimuli presented to rats during wakefulness elicited PGO_E from the pons (Fig. 2). No significant main effects or interactions were found in any of the ANOVAs, indicating that the 5-HT_{1A} agonist, 8-OH-DPAT, did not alter either the amplitude, latency, or proportion of PGO_E across six blocks of 40 tones.

Binding Studies

The results of the binding studies are summarized in Table 1. Quantitative autoradiographic analysis of [^{3}H]CN-IMI binding to 5-HT uptake sites indicated a moderate degree of innervation in PPT and relatively extensive innervation in LDT compared to the binding in the DRN, the major 5-HT cell body region. In contrast, [^{3}H]8-OH-DPAT binding to 5-HT_{1A} receptors in the PPT region revealed few receptor sites, whereas the LDT region revealed a moderate number of receptor sites relative to binding in the DRN.

TABLE 1

BINDING TO [^{3}H]CN-IMI AND [^{3}H]8-OH-DPAT IN DRN, PPT, AND LDT IN RATS

Region	N	[^{3}H]CN-IMI Binding (fmol/mg Protein)	N	[^{3}H]8-OH-DPAT Binding (fmol/mg protein)
DRN	6	6146 ± 344	8	1698 ± 103
PPT	8	843 ± 153	9	48 ± 17
LDT	11	3410 ± 175	10	500 ± 79

Values are mean ± SEM.

DISCUSSION

The ineffectiveness of locally injected 8-OH-DPAT in inhibiting PGO_E and the presence of relatively few 5-HT_{1A} receptor sites in PPT, as revealed by the present study, indicate that inhibitory modulation of PGO_E probably does not involve a 5-HT_{1A} receptor mechanism in PPT. These findings complement our extensive work in cats examining the role of 5-HT in the generation of spontaneous PGO waves. We have found no unequivocal evidence that a 5-HT_{1A} agonist locally microinjected into the region of cat PPT containing PGO wave "burst" neurons inhibits PGO wave generation. Neither the relatively specific 5-HT_{1A} agonist, 8-OH-DPAT, nor the less specific 5-HT_1 agonist, mCPP [1(3-chlorophenyl)piperazine], microinjected into the peribrachial region of PPT in cats significantly altered the frequency of PGO waves once REM was entered and maintained, although 8-OH-DPAT did reduce the number of successful entrances into REM (32).

Serotonin could influence the generation of PGO_E via a different receptor mechanism. A recent immunofluorescence study conducted in rats (25) found a very high degree of double labelling with antibodies for the 5-HT_2 receptor and for choline acetyltransferase in PPT and LDT, indicating that most 5-HT_2 receptors in this region were on cholinergic cells, and that most of these cells expressed the 5-HT_2 receptor. Thus, 5-HT_2 receptor mechanisms could be involved in the modulation of PGO waves. As yet, no one has examined the effects of local administration into PPT or LDT of 5-HT_2 drugs on sleep or on PGO waves in rats.

Many studies have demonstrated that manipulating 5-HT affects PGO waves in cats (2,5,14), presumably via a direct DRN influence on cholinergic cells in the pontine generator region (35). However, a preliminary study in cats found evidence of only a weak projection from DRN to LDT (28). Also, a recent study found relatively few serotonergic synapses on cholinergic neurons in PPT/LDT of the rat, and reported that the observed synapses were asymmetrical, a specialization most often associated with excitation, not inhibition (12). Although local injections of 8-OH-DPAT might have been effective if infused into LDT, the inability of Kayama et al. (17) to find bursting neurons in LDT suggests that it may not be an appropriate site in rats.

No one has yet considered the possibility that manipulations of 5-HT by various means may have their effects, not at the brain stem level, but at the level of the thalamus or the forebrain. A possible source of forebrain influence on PGO_E wave generation is the amygdala. The central nucleus of the amygdala (CNA) is known to project heavily to the parabrachial nucleus (24), a region with cellular activity linked to PGO waves in cats (4), and electrical stimulation of CNA in cats increases PGO waves during REM (3). We have found prelim-

inary evidence that a 1-mM concentration of the 5-HT antagonist methysergide, infused into the region of CNA, increases PGO waves in non-rapid eye movement sleep in rats (31), whereas microinjections of 5-HT terminate REM with short latency (33).

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