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THE ROLE OF 5-HT RECEPTOR SUBTYPES IN THE VENTROLATERAL ORBITAL CORTEX OF 5-HT-INDUCED ANTINOCICEPTION IN THE RAT

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Abstract—The present study examined the involvement of 5-HT in the ventrolateral orbital cortex (VLO) on descending antinociception and determined which subtypes of 5-HT receptors mediated this effect. This study focused on the effects of 5-HT microinjection in the VLO of lightly anesthetized male rats on the radiant heat–evoked tail flick (TF) reflex, as well as the influence of 5-HT_{1A}, 5-HT_2, 5-HT_3, and 5-HT_4 receptor subtype antagonists on the effect of 5-HT. Results showed that 5-HT microinjection (2, 5, 10 μg, in 0.5 μl) into the VLO depressed the TF reflex in a dose-dependent manner. Pretreatment with 5-HT receptor antagonists (1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine hydrobromide (NAN-190), cyproheptadine hydrochloride (CPT) and 1-methyl-N-(8-methyl-8-azabicyclo[3.2.3]-oct-3-yl)-1H-indazole-3-carboxamide maleate salt (LY-278,584)), specific for 5-HT_{1A}, 5-HT_2 and 5-HT_3 receptors, respectively, partially reversed the 5-HT-evoked inhibition. In contrast, the 5-HT_4 receptor antagonist, 1-[2-[(methylsulfonyl)-amino] ethyl]-4-piperidinyl]methyl1-methyl-1H-indole-3-carboxylate (GR 113808), had no effect on the inhibition of 5-HT. Microinjections of NAN-190, CPT and LY-278,584 alone into the VLO had no effect on the TF reflex. These results suggest that 5-HT_{1A}, 5-HT_2 and 5-HT_3, but not 5-HT_4 receptors, are involved in mediating 5-HT-induced antinociception in the VLO. According to different properties and distribution patterns of the 5-HT receptor subtypes on neurons, the possible mechanism of 5-HT activation of the VLO–periaqueductal gray (PAG) descending antinociceptive pathway is discussed. © 2008 Published by Elsevier Ltd on behalf of IBRO.

Key words: 5-HT, 5-HT receptor, ventrolateral orbital cortex, antinociception, tail flick reflex, rat.

Neuroanatomic studies have established that the spinal and medullary dorsal horn lamina I projects via the thalamic nucleus submedius (Sm) to the prefrontal ventrolateral orbital cortex (VLO), which contains neurons that project to the periaqueductal gray (PAG) (Craig and Burton, 1981; Hardy and Leichnetz, 1981; Craig et al., 1982; Yoshida et al., 1991, 1992; Coffield et al., 1992). The PAG is a region that is extensively involved in the modulation of nociception (Sandkühler and Gebhart, 1984; Fields and Basbaum, 1999). Studies in our laboratory have indicated that electrolytic lesion, or microinjection of GABA into the VLO, eliminates the Sm-mediated antinociception (Zhang et al., 1995a, 1998b, 1999). However, electrically or chemically-induced activation of the VLO depresses the spinal and trigeminal nocifensive reflexes, such as the tail flick (TF) reflex and the jaw-opening reflex. These antinociceptive effects are eliminated by lesion or a functional block of the PAG (Zhang et al., 1997a,b, 1998a). These findings suggest that the VLO is involved in an endogenous antinociception system, consisting of a spinal cord–Sm–VLO–PAG–spinal cord loop. Immunohistochemical studies have shown that a considerable number of 5-HT-producing neurons from the dorsal raphe nucleus (DRN) project to the VLO (Descarries et al., 1975; Matsuzaki et al., 1993). The 5-HT_{1A}, 5-HT_2, 5-HT_3, and 5-HT_4 receptors are distributed within the VLO (Kilpatrick et al., 1987; Chalmers and Watson, 1991; Pompeiano et al., 1994; Eglen et al., 1995; Hossein et al., 1996). However, it is not known whether 5-HT is involved in VLO-mediated antinociception, and if so, which 5-HT receptor subtypes participate in the 5-HT-induced antinociceptive effect. To answer this question, the present study examined the effects of 5-HT microinjected into the VLO on the TF reflex and also determined the influence of 5-HT_{1A}, 5-HT_2, 5-HT_3, and 5-HT_4 receptor antagonists on this effect in the rat. Some of the data reported here have been previously presented in abstract form (Qu et al., 2006).

EXPERIMENTAL PROCEDURES

Animal preparation

The following experiments were carried out on male adult Sprague–Dawley rats (n=58), weighing 220–300 g, provided by the Medical Experimental Animal Center of Shaanxi Province, China, and approved by the Institutional Animal Care Committee of the Xi'an Jiaotong University. The experimental protocol was in accordance with the ethical guidelines of the International Association for the Study of Pain (Zimmermann, 1983). All efforts were made to minimize the number of animals used and their suffering. The animals were anesthetized initially with sodium pentobarbital

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Abbreviations: AUC, areas under the curve; CPT, cyproheptadine hydrochloride; DRN, dorsal raphe nucleus; GR 113808, 1-[2-[(methylsulfonyl)-amino]ethyl]-4-piperidinyl]methyl1-methyl-1H-indole-3-carboxylate; LY-278, 584, 1-methyl-N-(8-methyl-8-azabicyclo[3.2.3]-oct-3-yl)-1H-indazole-3-carboxamide maleate salt; NAN-190, 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine hydrobromide; NRM, magnus raphe nucleus; PAG, periaqueductal gray; RM-ANOVA, repeated measures of analysis of variance; Sm, thalamic nucleus submedius; TF, tail flick; TFL, tail flick latency; VLO, ventrolateral orbital cortex.

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(50 mg/kg, intraperitoneally, SCRC, Shanghai, China). A tracheotomy and jugular vein cannulation were performed. The rat's head was positioned in a stereotaxic frame and a small craniotomy was performed over the prefrontal cortex, where a guide cannula (0.8 mm in diameter) was placed 2.0 mm dorsal to the VLO. The animals were maintained in a lightly anesthetized state with a constant, intravenous infusion (4–5 mg/kg/h) of sodium pentobarbital, which was sufficient to prevent spontaneous movements, yet allowed the TF reflex to be elicited (Sandkühler and Gebhart, 1984; Zhang et al., 1995a). Rectal temperature was monitored and kept between 37–38 °C by a thermostatically-regulated heating pad to prevent body temperature change elicited by anesthesia and drug injections (Hole and Tjolsen, 1993).

TF test

A heat-stimulation instrument (Tai-Meng Co., Chengdu, China) was used to apply radiant heat 5–6 cm from the tip of the tail to the blackened ventral surface of the tail to evoke the TF reflex. Tail flick latency (TFL) was manually measured with a stopwatch. The heat intensity was adjusted so that the baseline TFL was between 3.2–3.7 s; in order to prevent tail skin damage, a 7 s cutoff was employed. TFL was used as a measure of nociception and was repeatedly assessed with an inter-stimulus interval of 5 min. At least three baseline TFLs were obtained prior to intracerebral injection. Methods applied were as described previously (Sandkühler and Gebhart, 1984; Zhang et al., 1995, 1998).

Drugs

The drugs used in the present study included the following: 5-HT; 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine hydrobromide (NAN-190), a selective 5-HT_{1A} receptor antagonist; cyproheptadine hydrochloride (CPT), a 5-HT_2 receptor antagonist; 1-methyl-N-(8-methyl-8-azabicyclo[3.2.1]-oct-3-yl)-1H-indazole-3-carboxamide maleate salt (LY-278,584), a selective 5-HT_3 receptor antagonist; 1-[2-[(methylsulfonyl)-amino]ethyl]-4-piperidinyl]methyl1-methyl-1H-indole-3-carboxylate (GR 113808), a selective 5-HT_4 receptor antagonist. All drugs were purchased from RBI/Sigma Co., St. Louis, MO, USA. NAN-190 was dissolved in DMSO; all other drugs were dissolved in 0.9% normal physiological saline according to required dose. The volume of intracerebral injection for each drug was 0.5 μl. All drugs were freshly prepared prior to intracerebral injection.

Intracerebral microinjection

Via a guide cannula, 5-HT and/or 5-HT antagonists were microinjected into the VLO through a needle (0.4 mm in diameter) that was attached to a 1.0 μl Hamilton syringe. The needle protruded 2.0 mm beyond the guide cannula in order to approach the VLO according to atlas coordinates: 3.2–3.7 mm anterior to bregma, 2.0–2.5 mm lateral, 4.0–5.0 mm below cortical surface (Paxinos and Watson, 1986). Following establishment of a stable baseline TFL, 5-HT (2, 5, or 10 μg) was injected into the VLO over a period of 3 min. TFL was determined after injection termination and at 5-min intervals throughout a 65-min observation period. The same volume of vehicle (0.9% saline) was injected in the control animals.

The role of the 5-HT receptors in 5-HT-induced antinociception was assessed through the use of selective 5-HT_{1A}, 5-HT_2, 5-HT_3 and 5-HT_4 receptor antagonists (NAN-190 (10 μg), CPT (50 ng), LY-278,584 (2.5 μg), and GR 113808 (10 μg), respectively). The receptor antagonists were microinjected into the VLO 5 min prior to 5-HT (10 μg); TFL was obtained, subsequent to the antagonist microinjections and at 5-min intervals thereafter. The doses of the antagonists and agonists were chosen according to our preliminary experiments and to previous reports (Pozzi et al., 1995; Xiao et al., 1999, 2005).

In additional experiments, changes in TFL were assessed in response to 5-HT_{1A} (10 μg), 5-HT_2 (50 ng) and 5-HT_3 (2.5 μg) receptor subtype antagonists microinjected into the VLO alone. These experiments were performed at least 1.5 h after 5-HT injections in the animals that received 5-HT.

Histology

At the end of the experiment, the drug injection site was labeled with a Pontamine Sky Blue dye injection (0.5 μl, 2% in 0.5 M sodium acetate solution). Under deep anesthesia, the animals were transcardially perfused with 0.9% normal saline, followed by 10% formalin. The brain was removed and further fixed in 10% formalin solution for 7–10 days. The brain was cut in 30-μm thick sections with a freezing microtome, and the slices were stained with Cresyl Violet. The injection sites were plotted on the coronal sections modified from the Paxinos and Watson (1986) atlas. The diffusion range of 0.5 μl drug was 0.5 mm or less from the injection site, as previously reported (Zhang et al., 1998b).

Data analysis

All data were expressed as mean±S.E.M. Time course curves were performed and the areas under the curve (AUC) were measured for statistical analyses. The correlation between the dose and effect of 5-HT was analyzed using linear regression. One- or two-way repeated measures of analysis of variance (one- or two-way RM-ANOVA), together with post hoc multiple comparisons (Fisher LSD test), was used to assess the differences in entire observation time, as well as individual time points among different groups. A difference of $P<0.05$ was considered to be statistically significant.

RESULTS

As reported previously (Sandkühler and Gebhart, 1984; Zhang et al., 1995), stable TFL was maintained for more than 6 h in the lightly anesthetized rat. Baseline TFL was 3.4±0.02 s ($n=10$). Saline microinjection (0.5 μl) into the VLO had no effect on TF reflex; the mean TFL was 3.4±0.1 s ($n=6$) during the 65-min observation period.

Inhibitory effects of TF reflex following 5-HT microinjections into the VLO

A microinjection of 5-HT (2, 5, 10 μg) into the VLO depressed the radiant heat–induced TF reflex in a dose-dependent fashion ($r=0.999$, $P<0.001$). As shown in Fig. 1A, the time course curves from the treatment groups (i.e. saline and various doses of 5-HT-treated groups) were significantly different among treatments ($F_{(3,391)}=17.07$; $P<0.001$), times ($F_{(13,391)}=16.01$; $P<0.001$), and interactions ($F_{(39,391)}=5.28$; $P<0.001$). Further analyses showed that the inhibitory effects induced by 10 μg 5-HT on the TF reflex were significantly larger than the 5.0 μg 5-HT group ($P<0.001$). In addition, the effects of 5.0 μg 5-HT were significantly larger than the 2.0 μg 5-HT group ($P<0.05$). No significant difference was observed between the 2.0 μg 5-HT group and the saline group, as shown in Fig. 1B. Two microinjections of 5-HT (10 μg) into the VLO at an interval of 1.5 h in the same animal produced a similar inhibition of the TF reflex, as shown in Fig. 1C. The 5-HT injection sites in the VLO are shown in Fig. 1D.