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Peripheral 5-HT_{1B} and 5-HT_{2A} receptors mediate the nociceptive response induced by 5-hydroxytryptamine in mice

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ABSTRACT

While the role of 5-hydroxytryptamine (5-HT, serotonin) in the nociceptive processing has been widely investigated in the central nervous system, information regarding its role in peripheral tissues is still lacking. Noteworthy, 5-HT induces phenotypic changes of nociceptors and peripheral injection induces pain in humans and nociceptive response in rodents. However, local receptors involved in 5-HT effects are not well characterized. Thus, we aimed to investigate the role of 5-HT and some of its receptors in the peripheral nociceptive processing in mice. Intraplantar injection of 5-HT (10, 20 or 40 μg) into the hind-paw of mice induced paw licking behavior, which was inhibited by previous intraplantar treatment with cyproheptadine (5-HT_1 and 5-HT_2 antagonist; 0.5 or 5 μg), mianserin (5-HT_2 and 5-HT_6 antagonist; 0.1 μg), isamoltane (5-HT_{1B} antagonist; 0.5 or 5 μg) and ketanserin (5-HT_{2A} antagonist; 0.1 or 1 μg), but not by BRL 15572 (5-HT_{1D} antagonist; 1 or 10 μg), ondansetron (5-HT_3 antagonist; 1, 5, 10 or 20 μg) and SB 269970 (5-HT_7 antagonist; 2.5 and 25 μg). Altogether, these results indicate the local involvement of 5-HT_1, 5-HT_2 and 5-HT_6, especially 5-HT_{1B} and 5-HT_{2A}, in the nociceptive response induced by 5-HT in mice, thus contributing to a better understanding of 5-HT role in the peripheral nociceptive processing. In addition, they also point to important species differences and the need of a wide evaluation of the peripheral nociceptive processing in mice as these animals have been increasingly used in studies investigating the cellular and molecular mechanisms mediating the nociceptive response.

1. Introduction

5-Hydroxytryptamine (5-HT, serotonin) is found in high concentrations in enterochromaffin cells throughout the gastrointestinal tract and also in the central nervous system (CNS). Other important sources are platelets (in humans), mast cells (in rodents) and endothelial cells (Sanders-Bush and Mayer, 2006). Although 5-HT is involved in the regulation of many physiological processes and their malfunction, including those related to inflammation and pain (Hoyer et al., 2002), the exact sites and modes of its action are still being defined.

5-HT functions have been difficult to understand in part due to the high number of receptors present in different tissues coupled to different and sometimes opposing signal transduction systems. Signal transduction systems and structural diversity have been used as the basis for 5-HT receptors classification in seven families (5-HT_1 to 5-HT_7). 5-HT_1, 5-HT_2, and 5-HT_{4-7} receptor families are members of the superfamily of G protein-coupled receptors, while 5-HT_3 receptor is a ligand-gated ion channel (Barnes and Sharp, 1999; Palacios et al., 1990).

The involvement of 5-HT in the nociceptive processing has been investigated mainly in the CNS. The best characterized effect induced by 5-HT is the antinociception that results from its action at the spinal level after being released by neurons projecting from brain stem structures (Yaksh and Tyce, 1979; Yaksh and Wilson, 1979). Many receptors have been suggested to mediate the effect induced by 5-HT at the spinal level, including 5-HT_1, 5-HT_3 and 5-HT_7, among others (Liu et al., 1988; Kiefel et al., 1992; Lin et al., 1996).

The role of 5-HT in the peripheral nociceptive processing has also been investigated and a different profile is described. 5-HT induces phenotypic changes of nociceptors and there is wide support to its peripheral pronociceptive role. This amine acts as an inflammatory mediator that induces nociceptors sensitization (Taiwo and Levine, 1992). Peripheral injection of 5-HT induces pain in humans (Jensen et al., 1990) and paw edema (Cole et al., 1995) and nociceptive

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response in rats (Sufka et al., 1992; Taiwo and Levine, 1992). In addition, endogenous 5-HT is involved in the nociceptive response induced by subcutaneous (s.c.) injection of formalin in rats (Doak and Sawynok, 1997; Parada et al., 2001). Parada et al. (2001) demonstrated that 48/80 compound (a mast cell depleting agent) induces nociceptive response and cromoglycate (a mast cell degranulation inhibitor) inhibits the second phase of the nociceptive response induced by formalin in rats.

Different peripheral 5-HT receptors mediate the nociceptive response induced by 5-HT. Inhibition of 5-HT nociceptive response in rats by local injection of methysergide, ketanserin and ondansetron (Sufka et al., 1992; Taiwo and Levine, 1992; Tokunaga et al., 1998) indicates the involvement of 5-HT_1, $5HT_2$ and $5HT_3$ receptors. Furthermore, it has been shown that rat sensory neurons express 5-$HT_{1B,}$ 5-HT_{1D} e 5-HT_{2A} receptors (Chen et al., 1998), a result that supports the observation that thermal or mechanical sensitization induced by 5-HT in rats is inhibited by 5-HT_1 and 5-HT_2 antagonists (Taiwo and Levine, 1992; Tokunaga et al., 1998). The involvement of 5-HT_3 receptors in the peripheral nociceptive processing is also suggested, as selective 5-HT_3 antagonists inhibit nociceptive response induced by formalin and other inflammatory stimuli in rats (Giordano and Rogers, 1989; Okamoto et al., 2004).

Despite the wide knowledge about the role of 5-HT in nociception, most studies have focused its role in the CNS, while a clear information about its role in peripheral tissues is still lacking. In addition, many studies have identified 5-HT receptors or their corresponding mRNA in peripheral tissues of rats and have also investigated the nociceptive response induced by 5-HT and its pharmacological modulation in these animals, while such information is scarce for mice. In this context, the aim of this study was to investigate the role of peripheral 5-HT receptors in the nociceptive response induced by 5-HT in mice by using antagonists that target different receptors.

2. Material and methods

2.1. Animals

Male Swiss mice (25–30 g), with free access to food and water and maintained in a room with a 12 h light-dark cycle, were used. The experiments were carried out at 27 ± 1 °C, the thermoneutral zone for rodents. During the experiments, the animals were carefully handled, so as to result in the least behavioral stress. Intraplantar (i.pl.) injections were performed with the animal gently restrained in a soft piece of cloth, with the assistance of a second experimenter. Each animal was used once, being euthanized in a CO_2 chamber immediately after the experiment. The experiments were carried out according to ethical guidelines on animal experimentation (Zimmermann, 1983), and approved by the Ethics Committee on Animal Experimentation (CETEA) of the Federal University of Minas Gerais (protocol 132/2007).

2.2. Drugs and solutions

5-Hydroxytryptamine hydrochloride (Sigma, USA), cyproheptadine hydrochloride (Sigma, USA; 5-HT_1 and 5-HT_2 receptors antagonist), mianserin (Sigma, USA; 5-HT_2 and 5-HT_6 receptors antagonist), isamoltane hemifumarate (Tocris, USA; 5-HT_{1B} receptors antagonist), BRL 15572 (Sigma, USA; 5-HT_{1D} receptors antagonist), ketanserin tartrate (Biotrend, Switzerland; 5-HT_{2A} receptors antagonist), ondansetron hydrochloride (Cristália, Brazil; 5-HT_3 receptors antagonist) and SB-269970 hydrochloride (Sigma, USA; 5-HT_7 receptors antagonist) were used. All drugs, except BRL 15572, were freshly diluted in 0.9% NaCl. BRL 15572 was diluted in dimethylsulphoxide (DMSO) 4%.

2.3. Evaluation of nociceptive behavior

Nociceptive behavior was assessed after intraplantar (i.pl.) injection of different doses of 5-HT in a volume of 10 μl into the right hind-paw of mice. The time the animals spent licking the injected paw was determined within 20 min. Two or three observers evaluated the nociceptive behavior of the animals in the different experimental protocols. The observers were not aware of the treatments and registered the nociceptive behavior of animals from the different experimental groups within each protocol. In preliminary experiments, it was observed that after this period 5-HT-treated animals displayed nociceptive behavior that does not differ from that of saline-treated animals. To evaluate the role played by peripheral 5-HT receptors in the nociceptive response induced by 5-HT, different antagonists were injected ipsilaterally by the i.pl. route, in a volume of 10 μl, 10 min before 5-HT injection. To investigate whether the effect induced by the antagonists results from local or systemic actions, a contralateral-treated group of animals was added to the protocols.

2.4. Statistical analysis

The results were expressed as mean ± SEM. Comparison between groups was assessed by one-way analysis of variance followed by Newman–Keuls *post hoc* test. Differences with $p<0.05$ were considered significant.

3. Results

3.1. Paw licking behavior induced by 5-HT

I.pl. 5-HT injection in mice induced nociceptive response displayed as paw licking behavior, which started immediately after the injection. The doses of 2.5 and 5 μg did not induce response that differs from that induced by vehicle. The doses ranging from 10 to 40 μg induced intense paw licking behavior in mice, and the dose of 20 μg was chosen for subsequent protocols as it seems to induce maximal response that is not different from those induced by 10 or 40 μg of 5-HT (Fig. 1).

3.2. Effect induced by antagonists on paw licking behavior induced by 5-HT

To investigate the role played by peripheral 5-HT receptors in the nociceptive response induced by i.pl. injection of 5-HT (20 μg) in mice, different antagonists were injected (i.pl.) into the right hind-paw 10 min before 5-HT injection.

Previous treatment with cyproheptadine (0.5 and 5 μg; Fig. 2), mianserin (0.1 μg; Fig. 3), isamoltane (0.5 and 5 μg; Fig. 4) and ketanserin (0.1 and 1 μg; Fig. 5) partially inhibited paw licking behavior induced by 5-HT. When injected into the contralateral paw

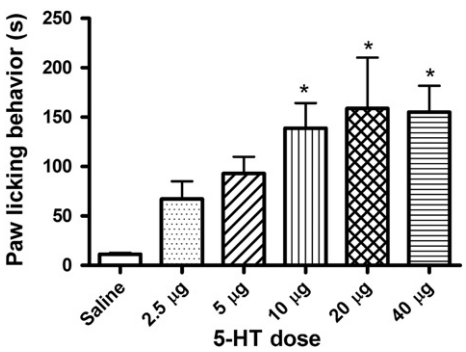

Fig. 1. Paw licking behavior induced by 5-HT (2.5; 5; 10; 20 or 40 μg/10 μl; i.pl.) in mice. *Statistical difference when compared to the group treated with vehicle, $p<0.05$ (n = 6–7).

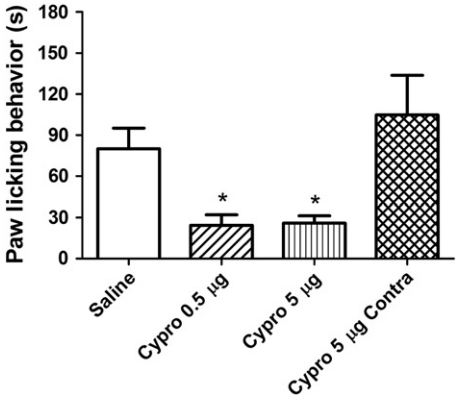

Fig. 2. Effect induced by cyproheptadine (0.5 or 5 μg; −10 min; 10 μl; i.pl.) on paw licking behavior induced by 5-HT (20 μg; 10 μl; i.pl.) in mice. *Statistical difference when compared to the group treated with vehicle, $p<0.05$ (n = 6).

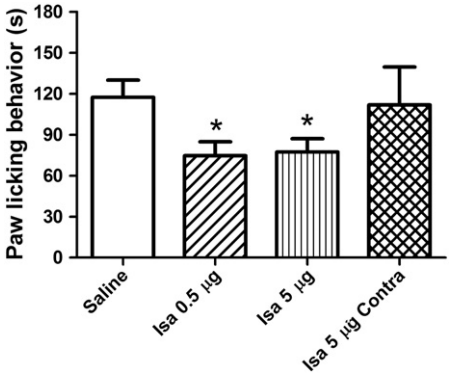

Fig. 4. Effect induced by isamoltane (0.5 or 5 μg; −10 min; 10 μl; i.pl.) on paw licking behavior induced by 5-HT (20 μg; 10 μl; i.pl.) in mice. * Statistical difference when compared to the group treated with vehicle, $p<0.05$ (n = 6–8).

and in the highest dose, these antagonists did not induce antinociceptive effect, suggesting that such effect may result from inhibition of 5-HT interaction with its peripheral receptors at the site of injection.

As the 5-HT_{1D} antagonist, BRL 15572, presents low solubility in saline, we used DMSO 4% to prepare its solution. Fig. 6A shows that previous (−10 min) i.pl. injection of DMSO 2 or 4% (10 μl, −10 min) did not change the nociceptive response induced by 5-HT. Although the nociceptive response induced by 5-HT was clearly inhibited by the previous antagonists, BRL 15572, at the doses used in the present study (1 and 10 μg), was devoid of such effect (Fig. 6B).

The 5-HT_3 and 5-HT_7 antagonists, ondansetron (5 and 20 μg; Fig. 7) and SB 269970 (2.5 and 25 μg; Fig. 8), respectively, also did not inhibit the nociceptive response induced by 5-HT. Even when co-injected, the antagonists ondansetron (1 and 10 μg) and SB 269970 (2.5 and 25 μg) also did not inhibit the paw licking behavior induced by 5-HT in mice (Fig. 9).

4. Discussion

Initially, we characterized the nociceptive response induced by 5-HT. I.pl. injection of this amine induced immediate paw licking behavior, that did not last long (approximately 20 min). Among the different experimental protocols, the magnitudes of the responses induced by 5-HT varied. As the protocols were carried out throughout the year, seasonal influences may be expected as these have been documented for other nociceptive stimuli (Winters et al., 1986; Perissin et al., 2003) and also for the antinociceptive effect induced by drugs (Winters et al., 1986). The concentrations of 5-HT in the injected solutions are much higher than the concentration of this amine found in the paw of rodents (Nitanda et al., 2005), raising the possibility of unspecific effects induced by 5-HT via interaction with targets different from the 5-HT receptors. However, the short duration effect induced by 5-HT is highly indicative of a fast inactivation and/or diffusion from the site of injection. Thus, the concentration of 5-HT in the injection solution certainly does not represent the real concentration of the amine in the tissue after its injection. In addition, previous studies have also demonstrated that doses of 5-HT higher than 30 μg are necessary to induce Fos expression in the dorsal horn neurons or nociceptive response in rodents (Doi-Saika et al., 1997; Parada et al., 2001; Tambeli et al., 2006; Tokunaga et al., 1998).

Several 5-HT receptors or their corresponding mRNA have been directly identified in sensory neurons of rats, including 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, 5-HT_4, 5-HT_{5A}, 5-HT_{5B} and 5-HT_7 (Chen et al., 1998; Sommer, 2004; Wu et al., 2001). In addition, increased mRNA levels for many of these receptors have been observed during inflammation induced by different stimuli in rats (Liu et al., 2005; Wang et al., 2003; Wu et al., 2001). Altogether, 5-HT receptors identification as well as increase of their expression indicates an important role played by 5-HT in peripheral features of inflammatory and nociceptive responses. Although, many studies have identified 5-HT receptors or their corresponding mRNA in peripheral tissues of rats, such information is scarce for mice.

Regarding 5-HT receptors function in the peripheral nociceptive processing, many aspects remain unclear as different roles have been attributed to different receptor subtypes. Some have attributed an inhibitory role to 5-HT_1 receptors, as i.pl. injection of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F} agonists inhibits the nociceptive response induced by formaldehyde in rats (Granados-Soto et al., 2010). These results are consistent with the frequently described signal

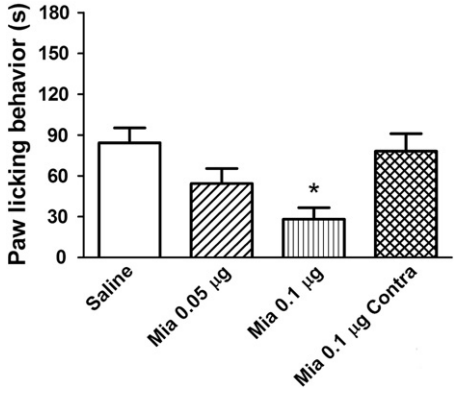

Fig. 3. Effect induced by mianserin (0.05 or 0.1 μg; −10 min; 10 μl; i.pl.) on paw licking behavior induced by 5-HT (20 μg; 10 μl; i.pl.) in mice. * Statistical difference when compared to the group treated with vehicle, $p<0.05$ (n = 10–13).

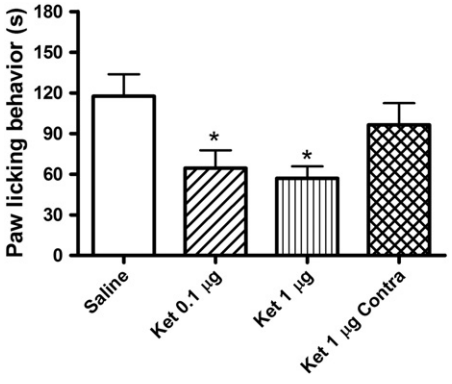

Fig. 5. Effect induced by ketanserin (0.1 or 1 μg; −10 min; 10 μl; i.pl.) on paw licking behavior induced by 5-HT (20 μg; 10 μl; i.pl.) in mice. * Statistical difference when compared to the group treated with vehicle, $p<0.05$ (n = 10–13).

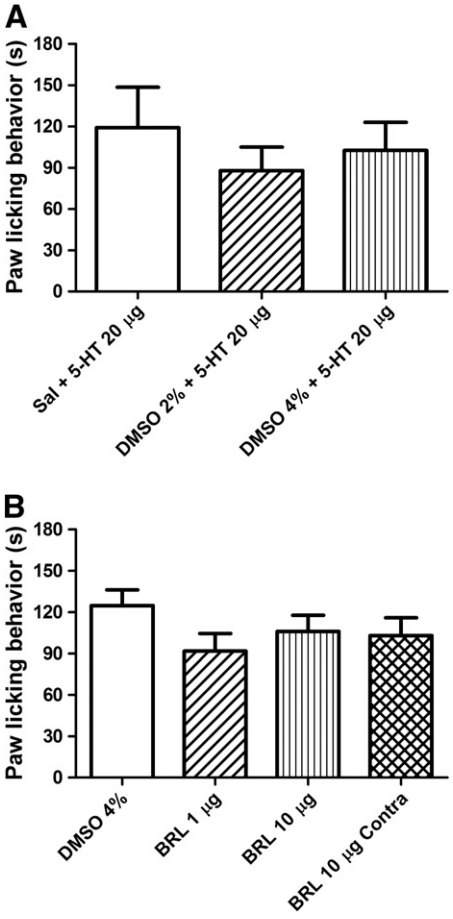

Fig. 6. Effect induced by DMSO (A −10 min; 10 µl; i.pl.) or BRL 15572 (B 1 or 10 µg; −10 min; 10 µl; i.pl.) on paw licking behavior induced by 5-HT (20 µg; 10 µl; i.pl.) in mice. (n = 4–14).

transduction system coupled to 5-HT_1 receptors, G_i/G_0 proteins that open potassium channels (Hannon and Hoyer, 2008; Hoyer et al., 1994).

However, despite the evidence suggesting an antinociceptive role for 5-HT_1 receptors, our results show that they also have a pro-nociceptive role, since isamoltane, a 5-HT_{1B} antagonist, and cyproheptadine, a 5-HT_1 and 5-HT_2 antagonist, inhibited paw licking behavior induced by 5-HT in mice. A peripheral role for these receptors in the response induced by 5-HT is clearly demonstrated by the lack of effect when the antagonists were injected into the contralateral paw. Corroborating our results, Taiwo and Levine (1992) showed that i.pl. injection of the 5-HT_{1A}

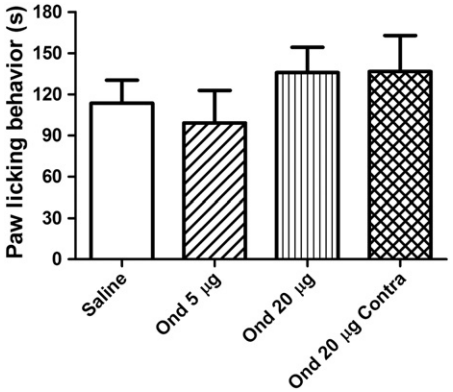

Fig. 7. Effect induced by ondansetron (5 or 20 µg; −10 min; 10 µl; i.pl.) on paw licking behavior induced by 5-HT (20 µg; 10 µl; i.pl.) in mice. (n = 6–7).

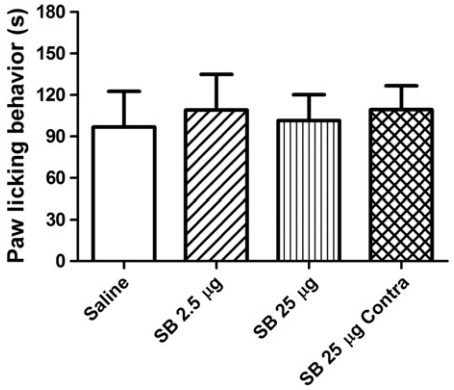

Fig. 8. Effect induced by SB 269970 (2.5 or 25 µg; −10 min; 10 µl; i.pl.) on paw licking behavior induced by 5-HT (20 µg; 10 µl; i.pl.) in mice. (n = 6–7).

antagonists spiroxatrine and spiperone attenuates the hyperalgesia induced by i.pl. injection of 5-HT in rats. Although Sufka et al. (1992) also concluded that peripheral 5-HT_1 receptors mediate the nociceptive response induced by i.pl. injection of 5-HT in rats, these authors used methysergide, a non-selective antagonist, thus limiting the association of this receptor to the observed response.

A challenge to the pronociceptive role of 5-HT_1 receptors is the inhibitory signal transduction system coupled to their activation, G_i/G_0 proteins that open potassium channels (Hannon and Hoyer, 2008; Hoyer et al., 1994). However, these receptors may also exert an excitatory role, since it has been found that 5-HT_{1B} activation is associated to inositol phosphate production and increase in intracellular calcium levels, leading to vascular smooth muscle contraction (Dickenson and Hill, 1995; Ebersole et al., 1993; Zgombick et al., 1993). Interestingly, in our study we observed that a selective 5-HT_{1B} antagonist, isamoltane, inhibited the nociceptive response induced by 5-HT. Altogether, these data suggest that the molecular mechanisms of second messengers generated upon activation of 5-HT_1 receptors may vary among different tissues and species, thus contributing to the different results reported in the literature.

The role of 5-HT_2 receptors, differently from that of 5-HT_1 receptors, in the peripheral nociceptive processing seems to be more clear-cut. We showed that ipsilateral, but not contralateral, i.pl. injection of ketanserin, a 5-HT_{2A} antagonist, inhibits paw licking behavior induced by 5-HT in mice. In spite of not being as straightforward as the effect induced by ketanserin, the effects induced by cyproheptadine and mianserin also provide evidence for

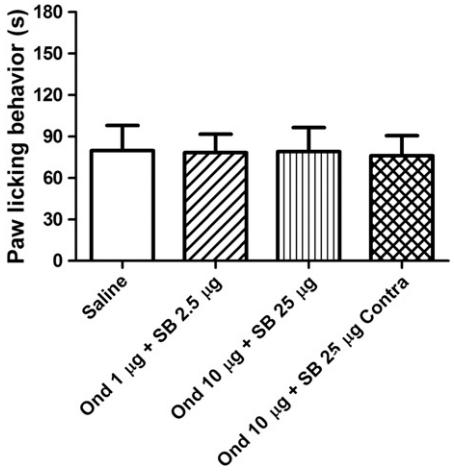

Fig. 9. Effect induced by co-administration of ondansetron (1 or 10 µg) and SB 269970 (2.5 or 25 µg) (−10 min; 10 µl; i.pl.) on paw licking behavior induced by 5-HT (20 µg; 10 µl; i.pl.) in mice. (n = 4–6).

the involvement of 5-HT_2 receptors in the response induced by 5-HT. Cyproheptadine and mianserin, in addition to interacting with 5-HT_2 receptors, also interact with 5-HT_1 and 5-HT_6 receptors, respectively.

In agreement with our results, ketanserin reduces thermal or mechanical hyperalgesia induced by various stimuli, including 5-HT, in rats only when applied over or injected into the ipsilateral paw (Hong et al., 2006; Wei et al., 2005). Sarpogrelate, another 5-HT_{2A} receptor antagonist, also inhibits the nociceptive response induced by formaldehyde and heat injury in rats only when administered into the ipsilateral paw (Obata et al., 2000; Sasaki et al., 2006). Considering that we and others have shown that the doses of ketanserin and sarpogrelate induced an effect only after ipsilateral injection, the set of results emphasizes the involvement of 5-HT_{2A} receptors in peripheral features of the investigated responses.

As the nociceptive response induced by i.pl. injection of 5-HT was immediate, it may result from direct activation of nociceptors, with rapid increases in membrane permeability to some ions, such as Na^+ and Ca^{+2}. Among the 5-HT receptors identified in sensory neurons that would expectedly mediate this immediate response is the 5-HT_3 receptor, an ion channel that allows rapid Na^+ and Ca^{+2} influx.

In spite of the presence of 5-HT_3 in sensory neurons (Chen et al., 1998; Sommer, 2004; Wu et al., 2001), we observed that its antagonist, ondansetron, did not inhibit paw licking behavior induced by 5-HT in mice. The role of 5-HT_3 receptors in the peripheral nociceptive processing is not well established. Tokunaga et al. (1998) and Oliveira et al. (2007) showed opposite results for the same 5-HT_3 antagonist, tropisetron, when tested against the nociceptive response induced by 5-HT in rats. Taiwo and Levine (1992) showed that i.pl. injection of 5-HT, but not of two 5-HT_3 agonists (2-methyl-5-hydroxytryptamine and phenylbiguanide), induces mechanical hyperalgesia in rats. In addition, an increase of Fos-like immunoreactivity in the spinal cord was observed after i.pl. injection of 5-HT and 5-HT_2 agonists, but not of 5-HT_3 agonists, in rats (Doi-Saika et al., 1997). These authors also showed that ketanserin (5-HT_{2A} antagonist), but not tropisetron, inhibited the response induced by 5-HT. There is no reasonable explanation for the discrepant data provided by all these studies. In the case of 5-HT_3 receptors, a species difference in the expression of this receptor subtype on sensory neurons cannot be pointed, as opposing results have been found even within the same species.

The role played by 5-HT_7 receptors in the peripheral nociceptive processing is even less well-known. 5-HT_7 receptors have also been identified in sensory neurons (Chen et al., 1998; Sommer, 2004; Wu et al., 2001) and couple to a signal transduction system that results in the increase of cAMP. Rocha-Gonzalez et al. (2005) showed that SB 269970, a 5-HT_7 antagonist, inhibits nociceptive behavior induced by formaldehyde, 5-HT or serotonergic agonists in rats. However, in our study we observed that the nociceptive response induced by 5-HT in mice was not inhibited by the same antagonist.

The absence of effect induced by ondansentron and SB 269970 on the nociceptive response induced by 5-HT in mice does not rule out the involvement of 5-HT_3 and 5-HT_7 receptors. One may wonder whether higher doses of both antagonists could induce an effect similar to that induced by other antagonists or that 5-HT_3 and 5-HT_7 receptors play a minor role. However, the absence of effect induced even by the association of ondansentron and SB 269970 indicates the absence of a peripheral role for 5-HT_3 and 5-HT_7 receptors in the nociceptive response induced by 5-HT in mice or, assuming that such a role exists, it is much reduced when compared to that of 5-HT_1 and 5-HT_2 receptors.

As many 5-HT receptors have been identified in sensory neurons and different peripheral sites, it is reasonable to assume that a single antagonist may not abolish 5-HT-induced responses, since this amine could interact with more than one excitatory receptor. Thus, the marked inhibition of 5-HT nociceptive response by cyproheptadine and mianserin may be related to their non-selective binding properties, targeting more than one receptor, which in turn contributes to a wide range effect. On the other hand, the inhibition of the nociceptive response by the selective antagonists' isamoltane and ketanserin indicates that 5-HT_{1B} and 5-HT_{2A} receptors have marked an involvement in the nociceptive response induced by i.pl. 5-HT in mice, even more important than that of other excitatory 5-HT receptors.

Some of our results differ from those reported by others who investigated the receptors mediating the nociceptive response induced by 5-HT. Although the use of different antagonists and different doses may explain the divergent results, the most likely possibility is difference between rats, used in most of the studies, and mice. Investigation of the nociceptive processing in the mouse and inherent species differences is warranted as its use is steadily increasing in the recent years due mainly to the wide availability of deficient and transgenic animals (Mogil, 2009).

5. Conclusion

In conclusion, we showed that i.pl. injection of 5-HT induces paw licking behavior in mice, which is inhibited by cyproheptadine, mianserin, isamoltane and ketanserin. Altogether, these results provide evidence of local involvement of 5-HT_1, 5-HT_2 and 5-HT_6, specially 5-HT_{1B} and 5-HT_{2A}, in the nociceptive response induced by 5-HT in mice, thus contributing to a better understanding of 5-HT role in the peripheral nociceptive processing. In addition, they also point to the need of a wide evaluation of the peripheral nociceptive processing in mice as these animals have been increasingly used in studies investigating the cellular and molecular mechanisms mediating the nociceptive response.

Conflict of interest

The authors state no conflict of interest.

Acknowledgments

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