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Receptor Mediation of 5-HT-Induced Inflammation and Nociception in Rats

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SUFKA, K. J., F. M. SCHOMBURG AND J. GIORDANO. *Receptor mediation of 5-HT-induced inflammation and nociception in rats.* PHARMACOL BIOCHEM BEHAV **41**(1) 53–56, 1992.—In light of evidence suggesting the proinflammatory and nociceptive action of peripheral serotonin (5-HT), the present study examined dose-dependent parameters of edema and algesia produced by intraplantar injections of 5-HT and the role of heterogeneous 5-HT receptors in these 5-HT-induced responses. Intraplantar 5-HT (0.05, 0.25, 0.5, or 1.0 μmol) produced paw edema at each 5-HT concentration and produced concentration-dependent increases in the nociceptive response as indexed by lifts of, and licks to the affected paw. Intraplantar pretreatment with the 5-HT_1 receptor antagonist methysergide at concentrations ≥3 nmol attenuated the 5-HT-induced (25 μmol) inflammatory and nociceptive responses. At concentrations ≥300 nmol, both 5-HT_2 receptor antagonist ketanserin and 5-HT_3 receptor antagonist odansetron pretreatment blocked 5-HT-induced inflammatory and nociceptive responses. These results more completely define peripheral 5-HT-receptor-dependent systems of 5-HT-induced inflammation and nociception in rats.

Serotonin 5-HT 5-HT receptors 5-HT antagonists Methysergide Ketanserin Odansetron
Inflammation Nociception Pain Edema Rats Analgesia Antinociception

THERE is literature to suggest the action of peripheral serotonin (5-HT) as a proinflammatory and pronocisponsive agent (1,11). During traumatic insult or pathologic changes that incur tissue damage, 5-HT from blood compartments has been shown to initiate and exacerbate inflammation (9,15). Moreover, both endogenous and exogenously applied 5-HT evoke nociceptive responses in the periphery (10,18). The mechanistic sequence by which 5-HT influences inflammation and nociception is unclear. Heterogeneous populations of 5-HT receptor types have been identified in the periphery (16). These receptor types may differentially mediate the inflammatory and algesic actions of 5-HT in peripheral tissues. One possibility is that peripheral 5-HT may act at 5-HT_1 and 5-HT_2 receptor types to potentiate vascular changes (2,17), thereby affecting the efflux of other pronociceptive and proinflammatory elements (e.g., bradykinins, eicosinoids) from the blood. Alternatively, plasma- and/or tissue-derived 5-HT may act at 5-HT_3 receptors localized to Aδ/C afferents to produce nociception (7,8). It is also feasible that these receptor systems may work concomitantly and/or synergistically to produce the noxious effects of 5-HT in the periphery. Thus the present research sought to more fully define the role of heterogeneous 5-HT receptors in 5-HT-induced inflammation and nociception by examining the differential ability of selective 5-HT receptor antagonists to alter these 5-HT-induced responses.

METHOD

Subjects

Male Sprague-Dawley rats (Dominion Laboratories, Omaha, NE) weighing 350–400 g were housed in pairs in suspended stainless steel cages (360 cm^2) at an ambient temperature of 23 ± 1°C, and were maintained on a 12-h light/dark cycle. Animals were allowed food, water, and conspecific contact ad lib prior to experimental use. All experiments were conducted during the middle ⅓ of the light cycle.

Apparatus

The observation chamber consisted of a 30 × 30 × 30-cm Plexiglas (0.75 cm) chamber with a hinged Plexiglas lid. The chamber floor was constructed of 2 × 2 hardware cloth. The observation chamber was raised 40 cm from the table, which permitted the placement of a 28 × 35-cm observation mirror. The mirror was fitted under the chamber floor at an angle of approximately 45°.

Drugs

All drugs were commercially obtained and were prepared daily before experimentation. In Experiment 1, 5-HT (0.05, 0.25, 0.5, and 1.0 μmol; Research Biochemicals Incorporated, Natick, MA) was intraplantarly (IPL) administered in a volume of 50 μl. In Experiments 2–4, the 5-HT_1 receptor antagonist methysergide (1, 3, and 10 nmol; RBI), the 5-HT_2 receptor antagonist ketanserin (30, 100, 300 nmol; RBI), and the 5-HT_3 receptor antagonist odansetron (100, 300 nmol and 1 μmol; Glaxo Pharmaceuticals, Research Triangle Park, NC) were IPL administered in a total volume of 25 μl, 5 min before IPL 5-HT administration. These 5-HT antagonist concentrations have been

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shown to be relatively selective for the respective 5-HT receptor type (14). In these antagonist studies, a 0.25-μmol 5-HT concentration was employed for induction of inflammatory nociception and was administered in an adjusted volume of 25 μl. In all cases, the temporal and dose parameters of 5-HT and 5-HT receptor antagonist drug effects were derived from pilot work in this laboratory. These initial studies also demonstrated that peripheral administration of 5-HT and 5-HT antagonists did not produce changes in motor abilities (i.e., limb flexion/withdrawal, body posture), respiration or affective responses (i.e., conspecific interactions, autogrooming) at any dose tested.

Procedure

In Experiment 1, rats were lightly anesthetized by a 2-min closed-septum exposure to methoxyflurane (Metofane) prior to IPL administration of 5-HT (0.05–1.0 μmol/50 μl) or saline into the plantar surface of the left hindpaw (n = 6 in all experiments). Following 5-HT injections, animals were placed into the observation chamber for 15 min. During this period, the number of lifts of and licks to the affected paw were recorded. Following the observation period, animals were placed into a small Plexiglas animal-restraining carrier and paw edema was assessed using a caliper to measure dorsoventral distance at the aponeuroisis to the nearest 0.5 mm.

Experiment 1 demonstrated that 5-HT concentrations ≥0.25 μmol produced significant edema and algesic responses. Thus this 0.25-μmol concentration of 5-HT was employed in subsequent studies (Experiments 2–4) that examined the ability of selective 5-HT receptor antagonists to alter patterns of 5-HT-induced edema and nociception. In these experiments, rats were pretreated with either the 5-HT_1 receptor antagonist methysergide (1–30 nmol), the 5-HT_2 receptor antagonist ketanserin (30–300 nmol), or the 5-HT_3 receptor antagonist odansetron (100 nmol–1 μmol) in a total volume of 25 μl. Antagonists were IPL administered 5 min before IPL 5-HT (0.25 μmol/25 μl) injections. All other parameters (i.e., dependent measures, observation period, apparatus) were as described in Experiment 1. Observations were performed by trained observers who were unaware of experimental treatment conditions.

Statistics

Data were analyzed using a one-way analysis of variance (ANOVA). Post hoc tests were performed using power-adjusted *t*-tests (13). In all cases, significance was considered at the level of $p<0.05$.

RESULTS

The results from Experiment 1 are summarized in Figs. 1 and 2. 5-HT produced edema at all concentrations tested (see Fig. 1). A one-way ANOVA of edema scores demonstrated a significant treatment effect, $F(4,25)=11.39$, $p<0.001$. Subsequent analyses of these data revealed that mean paw edema scores were significantly greater at all 5-HT concentrations (i.e., 0.05, 0.25, 0.5, and 1.0 μmol) compared to the mean edema score of saline control animals [$ts(25)=5.13$, 5.91, 4.54, and 5.32, respectively, all $ps<0.001$]. The lift and lick scores from Experiment 1 are summarized in Fig. 2. Intraplantar 5-HT produced concentration-dependent increases in the mean number of lifts and licks (see Fig. 2). One-way ANOVA revealed a significant treatment effect, $F(4,25)=31.57$, $p<0.001$. Subsequent analyses of lift/lick scores demonstrated significant algesic response at each 5-HT concentration (i.e., 0.05, 0.25, 0.5, and 1.0 μmol) as compared to the lift/lick score of the saline control group

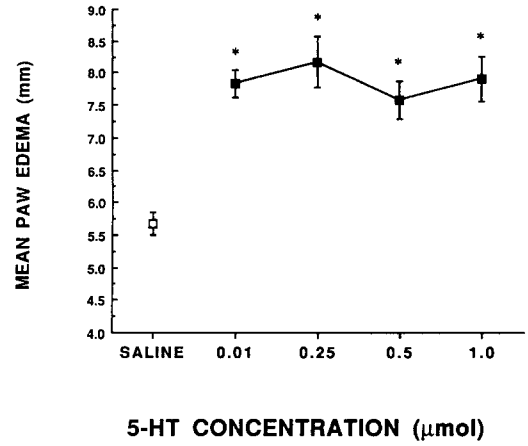

FIG. 1. The extent of paw edema produced by IPL 5-HT (0.01–1.0 μmol/50 μl) administration. Edema response was determined as the dorsoventral distance, as measured by calipers, 15 min after 5-HT administration. Values represent mean edema scores ± SEM for 6 determinations. *Indicates significant edema production as compared to the saline control animals, $p<0.05$.

[$t(25)=2.61$, 5.10, 6.24, and 10.66, respectively, all $ps<0.01$].

To permit comparison of the antagonist concentration-effect curves for methysergide, ketanserin, and odansetron, the data from these separate studies are summarized in Figs. 3 (edema scores) and 4 (lifts/licks). Edema produced by IPL 5-HT was differentially affected by the 5-HT_1, 5-HT_2, and 5-HT_3 receptor antagonists. When ranked by order of potency in attenuating edema, methysergide > ketanserin = odansetron. One-way ANOVA of the edema scores revealed a significant treatment effect of methysergide, $F(3,20)=22.79$, $p<0.001$, with significant reduction of 5-HT-induced edema produced by 3- and 10-nmol methysergide concentrations [$ts(20)=6.01$ and 6.87, respectively, $ps<0.001$]. As revealed by one-way ANOVA of the edema scores, ketanserin produced a significant treatment effect against 5-HT-induced paw edema, $F(3,20)=3.92$, $p=0.02$. Significant effects were observed at a ketanserin concentration of 300 nmol, $t(20)=2.79$, $p<0.01$. Odansetron produced a sig-

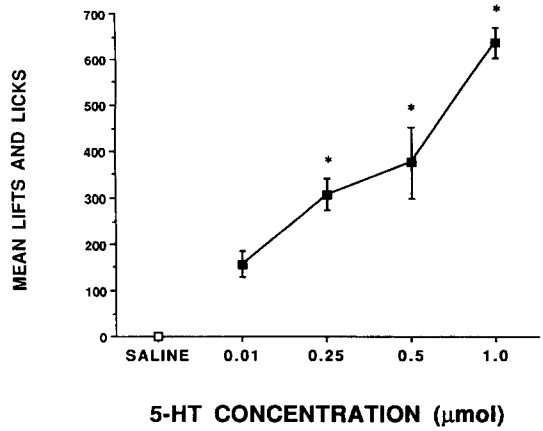

FIG. 2. The nociceptive effects of IPL administration of 5-HT (0.01–1.0 mol/50 μl). Values represent mean (±SEM, n = 6) lifts/licks to the affected paw for a 15-min observation period. *Indicates significant increase in lifts/licks as compared to the saline control animals, $p<0.05$.

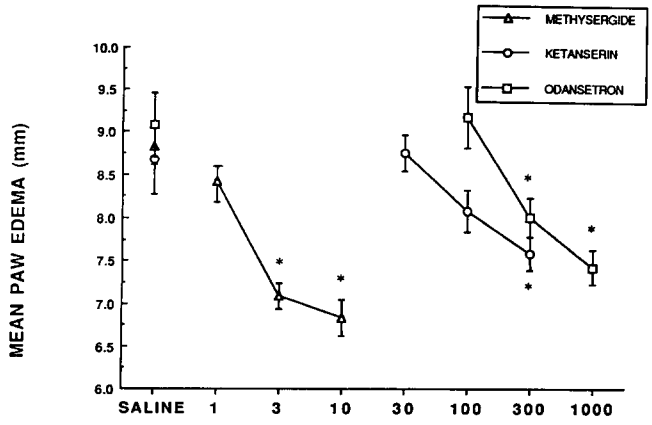

FIG. 3. The effects of 5-HT_1 antagonist methysergide (1–10 nmol), 5-HT_2 antagonist ketanserin (30–300 nmol), or 5-HT_3 antagonist odansetron (100 nmol–1.0 μmol) on paw edema produced by IPL 5-HT administration (0.25 μmol/ 25 μl). 5-HT receptor antagonists were administered 5 min before 5-HT administration. Edema response was determined as the dorsoventral distance, as measured by calipers, 15 min after 5-HT administration. Values represent mean edema scores ± SEM for 6 determinations. *Indicates significant edema prevention as compared to saline control animals, $p<0.05$.

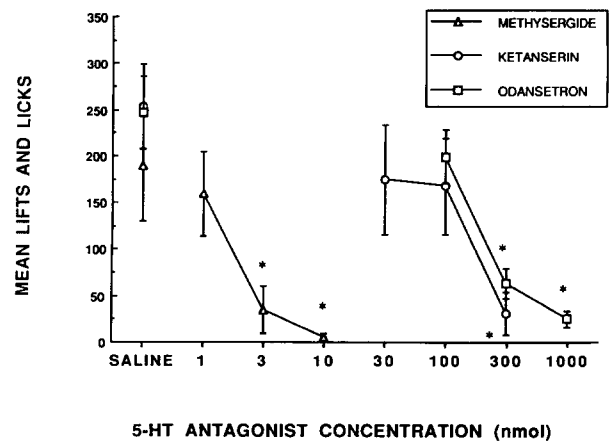

FIG. 4. The effects of 5-HT_1 antagonist methysergide (1–10 nmol), the 5-HT_2 antagonist ketanserin (30–300 nmol), and 5-HT_3 antagonist odansetron (100 nmol–1.0 μmol) on nociceptive responses produced by IPL 5-HT administration (0.25 μmol/25 μl). Values represent mean (± SEM, n = 6) number of lifts/licks to the affected paw for a 15-min observation period. *Indicates significant reduction of 5-HT-induced nociception as compared to animals receiving IPL 5-HT and saline, $p<0.05$.

nificant treatment effect against 5-HT-induced paw edema, $F(3,20)=8.11$, $p=0.001$, with significant edema reduction observed at the 300-nmol and 1-μmol concentrations [$ts(20)=2.55$ and 3.93, respectively, $ps<0.01$].

A summary of lift and lick scores from Experiments 2–4 is provided in Fig. 4. 5-HT-induced nociceptive responses (i.e., lifts and licks) were differentially affected by methysergide, ketanserin and odansetron. When ranked by order of potency to attenuate lifts/licks produced by IPL 5-HT, methysergide > ketanserin = odansetron. One-way ANOVA of lift and lick scores revealed a significant treatment effect of methysergide, $F(3,20)=5.32$, $p<0.001$, with significant attenuation of 5-HT-induced responses produced by 3- and 10-nmol concentrations [$ts(20)=2.77$ and 3.31, respectively, $ps<0.01$]. A one-way ANOVA of the nociception scores demonstrated that ketanserin produced a significant treatment effect, $F(3,20)=3.78$, $p=0.03$, with significant attenuation of 5-HT-induced nociception obtained at the 300-nmol concentration, $t(20)=3.31$, $p<0.01$. As revealed by one-way ANOVA, odansetron produced a significant treatment effect, $F(3,20)=16.51$, $p=0.0001$; attenuation of 5-HT-induced nociceptive responses were obtained at 300-nmol and 1-μmol odansetron concentrations [$ts(20)=4.96$ and 5.99, respectively, $ps<0.001$].

DISCUSSION

In agreement with previous reports (1, 9, 10), the present study demonstrated that exogenous 5-HT produced edema and nociceptive responses when microinjected at low concentrations to peripheral tissue. Multiple substrates may mediate 5-HT-induced inflammation and/or nociception. The lack of a relationship between extent of paw edema and the nociceptive response in Experiment 1 (see Figs. 1 and 2) may reflect differential recruitment of 5-HT systems acting on neuro- and vasogenic components of inflammatory pain. For example, 5-HT may act at the vasculature, inducing changes in vascular tone, to permit the extravasation of several identified proinflammatory and algesic substances. 5-HT may also stimulate peripheral mast cell degranulation to evoke the release of histamine and 5-HT to induce inflammation and nociception. As well, both of these phenomena may sensitize peripheral nocisponsive afferents to produce nociception and facilitate edema. It is unclear whether these processes occur concomitantly or sequentially. Our findings of the differential ability of selective 5-HT receptor antagonists to attenuate 5-HT-induced inflammation and nociception may provide insight to these processes.

The 5-HT_1 receptor antagonist methysergide was most effective in attenuating edema and nociception produced by 5-HT. Although ketanserin and odansetron significantly altered patterns of this 5-HT response, these agents were considerably less potent than methysergide. Given the specificity of methysergide, ketanserin, and odansetron, at the concentrations employed, to 5-HT_1, 5-HT_2, and 5-HT_3 receptors (14), respectively, it is likely that the observed results reflect discriminable involvement of heterogeneous 5-HT receptor types in 5-HT-induced inflammation and nociception. 5-HT_1 receptors have been localized to peripheral blood vessels (12). 5-HT acts at these sites to produce vasodilation (12), which may facilitate edema, and promote the efflux of prostaglandins, kinins, and other pronociceptive substances. Methysergide has been shown to exert antagonist action at several subtypes of the 5-HT_1 receptor (19). Thus the ability of this agent to attenuate 5-HT-induced inflammation and nociception may reflect its action at vascular 5-HT_1 receptors to antagonize these edematous and algesic processes. However, the differential involvement of these 5-HT_1 receptor subtypes in 5-HT-induced inflammation and nociception remain to be elucidated.

The effects of ketanserin against 5-HT-induced inflammation and nociception are somewhat more difficult to explain. 5-HT_2 receptors have been identified on the vasculature and serve to mediate contractile tone (2). Given this function, a role for vascular 5-HT_2 receptors in 5-HT-induced inflammation and nociception is unlikely. However, 5-HT_2 receptors have also been putatively identified on platelets and mast cells where they appear to mediate 5-HT release (3,4). We posit that the action of ketanserin likely reflects antagonism of platelet and mast cell 5-HT_2 receptor-mediated substrates in the cascade of 5-HT-in-

duced inflammatory and nociceptive processes.

The efficacy of odansetron in attenuating 5-HT induced inflammation and algesia is consistent with earlier work suggesting that peripheral 5-HT_3 receptors mediate a component of inflammatory nociception (7, 8, 18). 5-HT receptors have been identified on C-fiber primary afferents (5). Plasma-borne and/or mast cell-derived 5-HT may act at these sites to depolarize C-fibers and evoke a nociceptive response (7,8). Moreover, activated C-fibers antidromically release substance-P (6) to produce peripheral vasodilation contributing to the inflammatory response. The ability of odansetron to antagonize 5-HT-induced inflammation and nociception suggests inhibition of 5-HT_3 mediated C-fiber involvement in neurogenic inflammation.

In conclusion, it should be noted that, although the concentrations of 5-HT employed in the present research are greater than endogenous levels of 5-HT affecting peripheral tissues, this model is useful in evaluating possible mechanisms of 5-HT-induced inflammatory pain. The concentrations of 5-HT antagonists employed in these studies restrict their action to peripheral site of administration. Although it is possible that small concentrations may access central systems mediating nociception, such action is unlikely in light of the absence of observed centrally mediated 5-HT behaviors (i.e., changes in grooming, pseudoaffective responses, and body posture). This research suggests further assessment of 5-HT receptors mediating inflammation and algesia may reveal these sites to be pharmacologic targets for analgesic and anti-inflammatory therapeutics. As well, the demonstration of subtypes of 5-HT_1 receptors (e.g., 5-HT_{1A}, 5-HT_{1B}, etc.) suggests the possibility that these sites may differentially subserve components of the 5-HT_1 receptor-mediated responses. Furthermore, these studies suggest the involvement of other chemical substrates (e.g., eicosinoids, histamine, kinins) in 5-HT-induced effects in the periphery. Our laboratory is currently investigating these variables.

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