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Alterations of In-vitro 5-HT Receptor Pharmacology as a Function of Multiple Treatment with 5-Hydroxytryptamine or 8-Hydroxy-2-(di-*N*-propylamino) Tetralin in Rat Isolated Aorta, Uterus and Fundus, and Guinea-pig Isolated Trachea

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Abstract—The effects of single and multiple (5, 10, or 15 days) administration of the 5-HT_{1A} agonist 8-hydroxy-2-(di-*N*-propylamino) tetralin (8-OH-DPAT) (3 mg kg^{-1}, i.p.) or 5-HT (3 mg kg^{-1}, i.p.) in-vivo on the response of selected isolated smooth muscles to 5-HT in-vitro were investigated. Single dosing with 8-OH-DPAT did not alter the responses of the isolated tissues (rat aorta, uterus or fundus, or guinea-pig trachea) to 5-HT, while multiple dosing with 8-OH-DPAT produced rightward shifts and a suppression of the maximum response of all these tissues to 5-HT, with the exception of the rat stomach fundus. Multiple administration of 5-HT had no effect on the in-vitro response of the tissues to 5-HT. These data indicate that multiple administration of 8-OH-DPAT desensitizes or down-regulates the peripheral 5-HT_2 receptors found on the rat aorta and uterus, and guinea-pig trachea.

Cardiovascular and behavioural changes following subchronic administration with 8-hydroxy-2-(di-*N*-propylamino) tetralin (8-OH-DPAT), a 5-HT_{1A}-receptor agonist, have been previously described (Gradin et al 1985; Yamada et al 1988; Kolbasa et al 1991), and we have previously reported that acute or multiple (single daily dosing) administration of 8-OH-DPAT (3 mg kg^{-1}) produces differential changes in sensorimotor reactivity, clinical behaviour, and spontaneous activity levels following systemic administration in rats (Helton et al unpublished). Down-regulation of 5-HT_2 brain receptors has also been demonstrated (Peroutka & Snyder 1980; Leysen et al 1986; Conn & Sanders-Bush 1987) in rodents chronically treated with various antidepressants, 5-HT antagonists, and neuroleptics. However, the effects of multiple administration of 5-HT agonists have not been demonstrated in isolated smooth muscle tissues at the receptor level. The neurotransmitter, 5-HT, was evaluated as a reference comparator for 8-OH-DPAT. The dose examined (3 mg kg^{-1} 8-OH-DPAT or 3 mg kg^{-1} 5-HT) represents a dose which has been shown to produce changes in thermoregulation, gastrointestinal motility, satiety, or behaviour following systemic administration in rodents (Yamada et al 1988; Edwards & Stevens 1991; Jacoby et al 1991; Sugimoto et al 1991).

The present set of experiments was designed to investigate the effects of single (30 min pretreatment) and multiple (5, 10, or 15 consecutive days) treatment with 8-OH-DPAT or 5-HT in-vivo on the response of selected isolated smooth muscles, in-vitro.

Materials and Methods

Tissue preparations

Male and virgin female Sprague-Dawley rats, 200–300 g (Harlan Industries, Inc., Indianapolis, IN, USA), and male Hartley albino guinea-pigs, 280–560 g (Charles River Laboratories, Wilmington, MA, USA), were used.

Animals were administered 8-OH-DPAT (3 mg kg^{-1}, i.p.), 5-HT (3 mg kg^{-1}, i.p.), or physiological saline daily for 5, 10, or 15 days. Animals were killed and tissues removed 24 h after the last injection.

For experiments on rat aorta and guinea-pig trachea smooth muscle, animals were killed by cervical dislocation, and the tissues were removed. The aorta and trachea were then cut into 4–5 mm rings and attached with thread to a stationary glass rod and the tissue placed in an organ bath containing Krebs bicarbonate solution. The other end of the thread was attached to a force-displacement transducer. The tissues were placed under a resting tension of 2 g.

For experiments on rat fundus, animals were killed by cervical dislocation, and the stomach was removed. The fundus was opened into a sheet and cut to form a large strip which was then cut into a smaller strip. One end of the fundal strip was attached to a glass rod and the other end to a force-displacement transducer. The tissues were then placed into organ baths containing Krebs bicarbonate solution. The tissues were placed under a passive force of 4 g. For experiments on rat uterus, the animals were killed by cervical dislocation and the abdomen opened. A section of one uterine horn was removed from each animal. Each segment was then attached to a glass rod with thread and placed into organ baths containing DeJalon's solution. The other end was attached to a force-displacement transducer. The tissues were placed under a passive force of 1 g.

Isolated tissues were placed in either Krebs bicarbonate or DeJalon's solution (rat uterus) and allowed to equilibrate for 1–2 h at their assigned resting tensions before exposure to drugs. Tissue-bath solutions were maintained at 37°C room temperature (21°C) (rat uterus) and aerated with 95% O_2–5% CO_2. Isometric measurements were made with Grass FT03 transducers and recorded on a Beckman

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Dynograph Model R-611. In all experiments, only one tissue was removed from each animal to achieve a population of four for each treatment group.

Bioassay

Single treatment experiments. Concentration-response curves were generated with 5-HT in each of the tissue types. After a wash-out period (20–25 min), concentration-response curves to 8-OH-DPAT were generated. The highest concentration of 8-OH-DPAT (10^{-5} M) was allowed to remain in contact with the tissues for 30 min. The tissues were again washed, and a concentration-response curve generated with 5-HT.

Multiple treatment experiments. Concentration-response curves were generated with 5-HT in each of the different tissues from animals treated subchronically for 5, 10, or 15 days with 8-OH-DPAT, 5-HT or physiological saline.

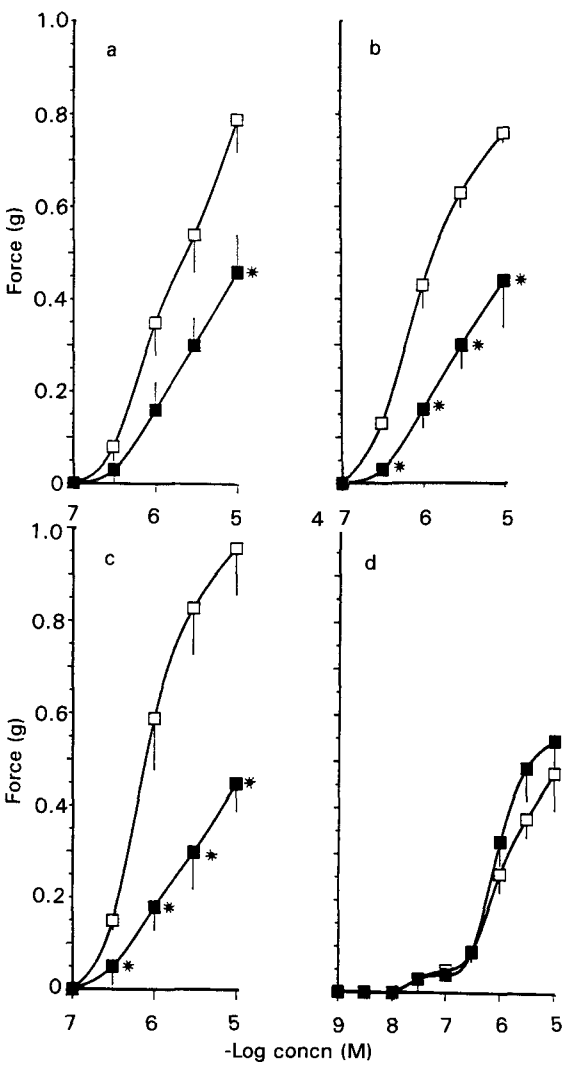

FIG. 1. The effects of multiple administration of 8-OH-DPAT or 5-HT in-vivo (3 mg kg^{-1}, i.p.) on the contractions of the rat aorta induced by 5-HT in-vitro. a. 5 days (8-OH-DPAT ■, saline □); b. 10 days (8-OH-DPAT ■, saline □); c. 15 days (8-OH-DPAT ■, saline □); d. 15 days (5-HT ■, saline □). Each point represents the mean ± s.e. (n + 4).

Drugs and related compounds

The solutions of 8-OH-DPAT (3 mg mL^{-1}) and 5-HT (3 mg mL^{-1}) used for daily dosing were prepared in physiological saline. The solutions of 8-OH-DPAT and 5-HT for use in the isolated tissue experiments were prepared fresh in Milli-Q water. Dilutions of the stock solutions to achieve tissue bath concentrations were made in the appropriate physiological solution. 5-Hydroxytryptamine creatinine sulphate was from Sigma Chemical Company (St Louis, MO, USA) and 8-hydroxy-2-(di-*N*-propylamino) tetralin was from Research Biochemicals Inc. (Natick, MA, USA). All solutions were prepared fresh daily.

Statistical analysis

Results were expressed as the mean ± s.e. For the acute experiments, statistical differences between tissues treated with 8-OH-DPAT or saline were determined by Student's *t*-test for unpaired groups (Haber & Runyon 1977). For the subchronic experiments, statistical differences between tissues from animals treated with multiple (5, 10, or 15 days) 5-HT or 8-OH-DPAT and saline were determined by Student's *t*-test for unpaired data. Statistical significance was set at $P < 0.05$.

Results

Single treatment with 8-OH-DPAT (10^{-5} M) did not alter the responses of the rat isolated aorta, uterus or fundus or guinea-pig trachea to 5-HT in-vitro (data not shown).

In the rat aorta, multiple dosing with 8-OH-DPAT for 5 and 10 days in-vivo, produced a rightward shift and

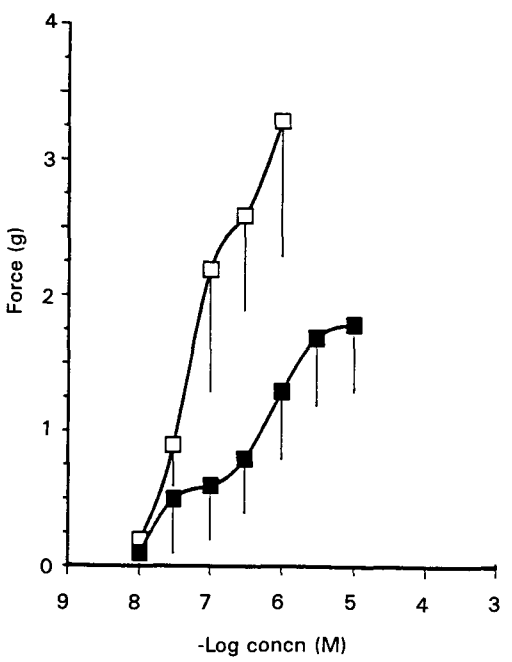

FIG. 2. The effects of multiple (15 day) administration of 8-OH-DPAT in-vivo (3 mg kg^{-1}, i.p.) on the contraction of the rat uterus induced by 5-HT in-vitro. Each point represents the mean ± s.e. (n = 4). ■ 8-OH-DPAT, □ saline.

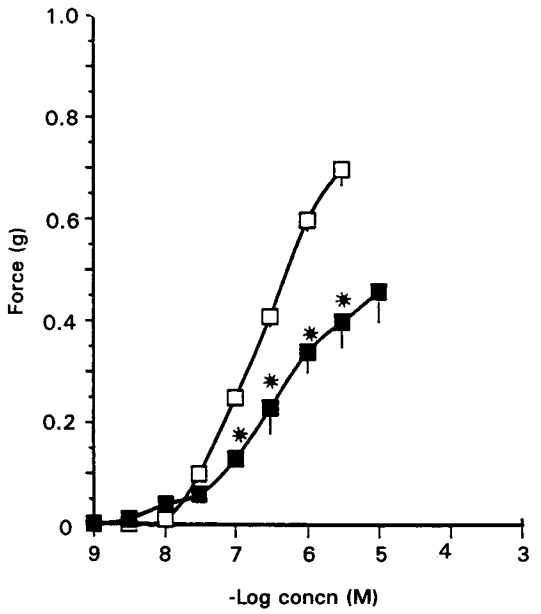

FIG. 3. The effects of multiple (15 day) administration of 8-OH-DPAT in-vivo (3 mg kg^{-1}, i.p.) on the contraction of the guinea-pig trachea induced by 5-HT in-vitro. Each point represents the mean ± s.e. (n = 4). ■ 8-OH-DPAT, □ saline.

suppression of the maximum response to 5-HT in-vitro (Fig. 1a,b) as compared with those tissues taken from animals treated with saline for the same period. Five-day treatment with 8-OH-DPAT in-vivo produced a 4-fold shift of the response of the aorta to 5-HT in-vitro, and 10-day treatment produced approximately an 8-fold shift in-vitro. The response of the tissues (from animals treated with 8-OH-DPAT in-vivo) to 5-HT was approximately 58% of the maximum response to 5-HT (from animals treated with saline in-vivo).

Rat aorta from animals treated for 15 days with 8-OH-DPAT in-vivo responded approximately 47% of the maximum response to 5-HT in-vitro (as compared with saline-treated animals). Treatment with 8-OH-DPAT for 15 days in-vivo also resulted in an apparent 21-fold shift in the response of the aorta to 5-HT in-vitro (Fig. 1c). Multiple treatment with 5-HT for 15 days in-vivo did not alter the response of the aorta to 5-HT in-vitro (Fig. 1d).

For rat uterus, multiple treatment with 8-OH-DPAT for 15 days in-vivo produced approximately a 40-fold rightward shift of the response of the uterus to 5-HT in-vitro (approx. 55% of the maximum response of 5-HT from animals treated with saline) (Fig. 2). Multiple treatment for 15 days with 5-HT in-vivo did not alter the response of the uterus to 5-HT in-vitro (data not shown).

For guinea-pig trachea, tissues from animals treated for 15 days with 8-OH-DPAT in-vivo, there was a suppressed response to 5-HT in-vitro (−34%) and a shift to the right (6-fold) of the response-curve as compared with saline-treated animals (Fig. 3). However, tissues from animals treated for 15 days with 5-HT (3 mg kg^{-1}, i.p.) or saline in-vivo responded similarly to 5-HT in-vitro (data not shown).

Multiple treatment for 15 days with 8-OH-DPAT or 5-HT in-vivo did not effect the response of the rat stomach fundus to 5-HT in-vitro as compared with tissues from saline-treated animals (data not shown).

Discussion

The 5-HT receptors of the rat aorta, uterus, and guinea-pig trachea have previously been reported to be of the 5-HT$_2$ subtype. The 5-HT receptors responsible for contractile activity in the rat isolated stomach fundus have been shown to differ from those already described (Cohen et al 1981; Van Nueten et al 1982; Wrigglesworth 1983).

Multiple treatment with 8-OH-DPAT in-vivo produced rightward shifts as well as a suppression of the maximum response to 5-HT in the rat aorta and uterus, and in guinea-pig trachea. However, multiple treatment with 8-OH-DPAT in-vivo did not alter the response of the rat isolated stomach fundus to 5-HT. Similar multiple dosing experiments conducted with 5-HT in-vivo did not produce any changes in the response of these isolated tissues to 5-HT.

Classically, when tissues are over-exposed to an agonist, a decrease in the response to that agonist is noted. This phenomenon is generally described as down-regulation (Axelson & Thesleff 1958). The results of these experiments indicate that multiple dosing with the 5-HT$_{1A}$ agonist, 8-OH-DPAT, produces either desensitization or down-regulation of the peripheral 5-HT$_2$ receptors found on these tissue types.

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