ABSTRACT # 768

Piboserod (SB 207266), a selective 5-HT$_4$ receptor antagonist, reduces serotonin potentiation of neurally-mediated contractile responses of human detrusor muscle

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ABSTRACT

Introduction and Objective: 5-HT has been shown to enhance the contractions induced by electrical field stimulation (EFS) of isolated human detrusor strips thereby facilitating the neuromuscular parasympathetic cholinergic transmission. The 5-HT receptor mediating this potentiating effect has been identified as the 5-HT$_4$ subtype located prejunctionally on the cholinergic terminations within the detrusor wall. The goal of this study was to evaluate the potency of piboserod (SB 207266), a highly potent and selective 5-HT$_4$ receptor antagonist, at inhibiting the potentiating effect of 5-HT on human detrusor contractions.

Methods. Strips of human detrusor muscle were mounted in Krebs-HEPES buffer under resting tension of 500 mg and EFS (20 Hz, 1 ms duration at 300 mA for 5 s) was applied at 1-min intervals and allowed to equilibrate. Concentration-response curves to 5-HT (0.1 nM - 100 mM) were performed in absence or in presence of 1 or 100 nM of piboserod. (n=3-4 experiments with n=3-4 different bladders samples per condition). The experiments were performed in presence of methysergide (1 mM) and ondansetron (3 mM) to block 5HT$_1$/5HT$_2$ and 5-HT$_3$ receptors, respectively.

Results. As previously described, 5-HT potentiated the contractile response to EFS in human bladder strips in a concentration-dependant manner, with a maximum mean of 60 ± 19.9 % of the basal EFS-evoked contractions. Piboserod did not modify basal contractions (before 5-HT) but induced a concentration-dependant antagonism of the bladder strips response to 5-HT. This translated into a rightward shift of the concentration-response curve with a reduction of the maximal response to 5-HT. In presence 1 and 100 nM of piboserod, the maximal potentiation of the EFS-evoked contractions with 5-HT were 45.0 ± 7.9 and 38.7 ± 8.7 %, respectively. **Conclusion.** These data show the ability of piboserod to antagonize with high potency the facilitator properties of 5-HT on neurogenic contractions of isolated human bladder strips. The 5-HT$_4$ receptor might represent an attractive therapeutical target for the treatment of overactive bladder.

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INTRODUCTION & OBJECTIVE

- Beneficial effects of the gastrointestinal prokinetic 5-HT$_4$ agonist cisapride have been reported on voiding disorders in patients with urinary retention due to acontractile bladder after spinal cord injury [1,2]. Additionally, an increase in micturition frequency has been described in patients treated with cisapride for gastrointestinal motor disturbances [3,4].
- In vitro, 5-HT has been shown to enhance the contractile responses to electrical field stimulation (EFS) of isolated human detrusor strips by facilitating the neuromuscular parasympathetic cholinergic transmission [5]. The 5-HT receptor mediating this facilitator effect has been pharmacologically identified as the 5-HT$_4$ receptor subtype located prejunctionally on the cholinergic terminations within the detrusor wall [6,7].
- To our knowledge, there is no available clinical data regarding the effect of a 5-HT$_4$ antagonists on bladder function. Nevertheless, considering the properties of 5-HT$_4$ agonists, a selective 5-HT$_4$ receptor blockade may have a potential role in the treatment of overactive bladder for which a decrease of cholinergic stimulation is expected to be beneficial [8].
- Piboserod (SB-207266) is a highly and potent selective 5-HT$_4$ receptor antagonist for which studies in isolated human intestine have estimated an apparent K_B value of 0.1 nM [9].
- The present study evaluates the potency of piboserod at human bladder 5-HT$_4$ receptors in support of its potential usefulness for the treatment of overactive bladder.

METHODS

Human detrusor strips preparation

Samples of human bladder were obtained from patients undergoing cystectomy for infiltrating bladder cancer (57 ± 2.5 years old). All bladder samples were collected with patient informed consent according to local regulation. Only samples from donors with no known bladder dysfunction were used. A macroscopically normal part of the dome with no tumoral tissue was selected for experiments and detrusor strips (5 x 2 mm) were prepared by removing serosal and mucosal layers.

Strips were mounted isometrically at a resting tension of 500 mg in 5 ml organ baths filled with Krebs-HEPES buffer maintained at 37°C and continuously bubbled with 95%O_2-5%CO_2. The strips were connected to force transducers and the tension changes were recorded.

Characterization of the response to electrical field stimulation (EFS)

Experiments were performed in presence of 1 µM of methysergide (a 5-HT$_1$/5-HT$_2$ receptor antagonist) and 3 µM of ondansetron (a selective 5-HT$_3$ receptor antagonist) to isolate pharmacologically the 5-HT$_4$ receptor subtype.

EFS was applied with the following parameters : 20 Hz, 1 ms pulse duration, 5-s train at 300 mA delivered at 1-min intervals. When stable contractile responses were obtained, cumulative concentration-response curves to 5-HT were determined. Each concentration remained in contact with the tissue until a stable response was reached. Following completion of the first 5-HT concentration-response curve, the strips were washed for 1 hour, with continuation of EFS. Piboserod (1 nM or 100 nM) or vehicle (water) was then incubated with strips, and following a 30-min equilibration period with continued EFS, a second 5-HT concentration-response curve was then determined.

RESULTS

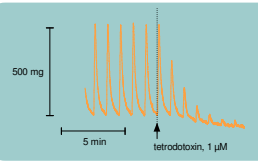

Figure 1 : Illustration of the effect of tetrodotoxin (1µM) on EFS-evoked isolated human bladder strip contractions. EFS elicited reproducible twitch contractile responses of the isolated human bladder strips that were markedly inhibited after exposition to tetrodotoxin (1 µM), thus confirming that they were due to the recruitment of neural terminations.

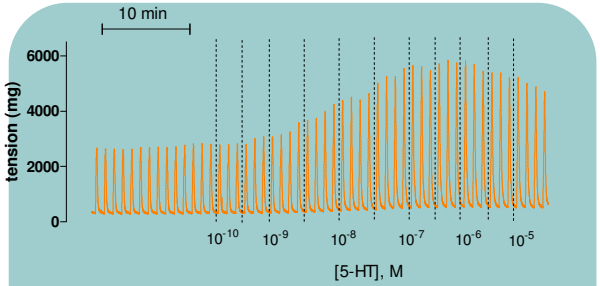

Figure 2 : Representative recording of EFS-evoked twitch contractions of isolated human bladder strips exposed to cumulative concentrations of 5-HT.

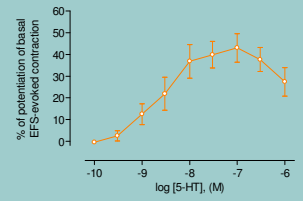

Figure 3 : Potentiating effect of 5-HT on the contractile response of human isolated bladder strips. First concentration-response curve prior to the exposure to piboserod or vehicle. Values are mean ± SEM of n= 4 bladders.

- Cumulative addition of 5-HT produced a biphasic effect on EFS-evoked contractions of bladder strips. The maximal response to 5-HT corresponded to an increase of the basal contractions of 43.0 ± 6.6 % with an pEC$_{50}$ value of 8.4 ± 0.7 (Figures 2 and 3).

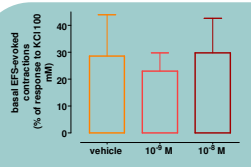

Figure 4 : Basal EFS-evoked twitch contractions in presence of vehicle, 1 nM and 100 nM of piboserod. The cntractions were not changed by the presence of the antagonist (n=4, p=0.92, one-way ANOVA).

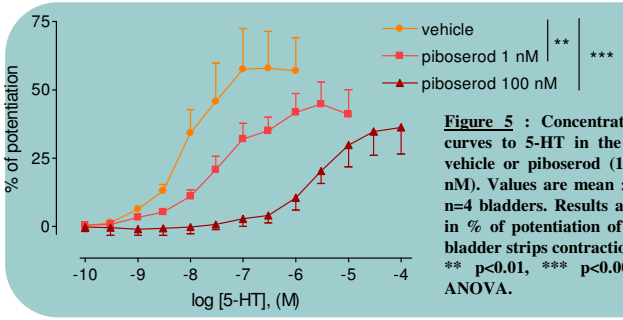

Figure 5 : Concentration-response curves to 5-HT in the presence of vehicle or piboserod (1 nM or 100 nM). Values are mean ± SEM from n=4 bladders. Results are expressed in % of potentiation of EFS-evoked bladder strips contractions. ** p<0.01, *** p<0.001, two-way ANOVA.

- Piboserod significantly altered the response of bladder strips to 5-HT (Figure 5). Indeed, piboserod induced a concentration-dependent antagonism translated into a rightward shift of the curves. The maximal responses to 5-HT were reached at 3 and 100 µM in presence of 1 and 100 nM of piboserod, respectively and pEC$_{50}$ values of 7.4 ± 0.3 and 5.5 ± 0.3 were obtained at these respective concentrations of antagonist (n=4) (Piboserod 100 nM vs vehicle : p<0.001, one-way ANOVA followed by a Newman-Keuls multiple comparison test).

CONCLUSION

- This study confirms the enhancer effect of 5-HT on the neurally-mediated contractile response of normal human bladder strips and it shows the ability of piboserod, a 5-HT$_4$ receptor antagonist, at antagonizing this potentiating effect.
- In absence of 5-HT, piboserod had no effect on EFS-evoked bladder strip basal contractions, suggesting a lack of 5-HT$_4$ receptor-mediated tone and no intrinsic 5-HT$_4$ receptor activity in normal human bladder.
- Nevertheless, the question that remains to answer is whether the endogenous 5-HT levels and/or the ability of 5-HT to enhance cholinergic neuromuscular transmission are increased in human idiopathic overactive bladder (OAB). Whether piboserod could alter the 5-HT/5-HT$_4$ pathway-mediated facilitation of neurotransmission in idiopathic OAB will require further investigations. This should provide a key argument for supporting the clinical exploitation of a 5-HT$_4$ receptor antagonist such as piboserod for the management of voiding disorders associated with OAB, for which a decrease of cholinergic stimulation is expected to be beneficial.