

Effects of besipirdine and its main metabolite on the contractility of the rabbit isolated urethra

Stefano Palea¹, Moèz Rekik¹, Jacques Fertè², Hugues Bienaymé² & Philippe Lluel¹

¹UROsphere, Toulouse, France ²UroGene, Paris, France

AIMS OF THE STUDY

Besipirdine hydrochloride is currently undergoing clinical trials in Europe and Australia, in patients suffering from overactive bladder. The effects of besipirdine and its main metabolite (HP748) on the adrenergic system are well documented (1). It is well known that α_1 -adrenoceptors are implicated in the contractility of the mammalian urethra. However, the effects of besipirdine and HP748 on the isolated urethra were never assessed. The aim of this study was therefore to evaluate the functional activities of the 2 compounds, in relation with their norepinephrine (NE) reuptake inhibition and α_1 -adrenoceptor agonism properties.

MATERIALS & METHODS

Adult New Zealand female rabbits were killed by cervical dislocation and exsanguinated. Two smooth muscle strips were obtained from the medial and distal urethra, respectively, and mounted in organ baths containing a Krebs solution (pH 7.4, gassed with 95% O₂ and 5% CO₂ at 37°C). Propranolol (1 μ M), normetanephrine (1 μ M) desipramine (0.1 μ M) and deoxycorticosterone (3 μ M) were added to the Krebs solution in order to block β -adrenoceptors, catechol-O-methyltransferase and the uptake 1 and 2, respectively. Contractile responses were measured using isometric tension transducers (Grass FT03) and recorded using a MacLab 8e data acquisition system. An initial tension of 1 g was applied. After 60 min of equilibration, urethral strips were exposed twice to NE (30 μ M), at 60 min interval. Strips having a contractile response < 1 g were discarded.

Protocol 1 (NE reuptake inhibition): Following a 30 min washout period, a cumulative NE concentration-response curve (CRC) in the range 0.01-100 μ M was performed. After a new 30 min washout period, besipirdine or tomoxetine (both at 1 μ M) were incubated for 30 min, then a second CRC to NE was completed. The results were expressed as pEC₅₀ values for the second CRC to NE in the presence of test substances or their solvent (distilled water).

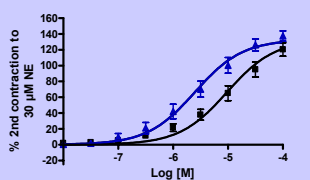
Protocol 2 (α_1 -adrenoceptor agonism): Following a 30 min washout period, organ baths were challenged with HP748, added in cumulative concentrations in the range 0.01-100 μ M, in the absence or presence of 1 μ M prazosin. Moreover, a time-matched control curve to the solvent (DMSO) was generated in parallel. Besipirdine was also tested using the same protocol. Results were expressed as % of the contraction induced by the second challenge with 30 μ M NE.

RESULTS

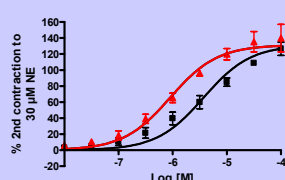
Protocol 1: NE reuptake inhibition

Effect of test compound at 1 μ M on the concentration-response curve to norepinephrine in the rabbit isolated urethra

A. Besipirdine 1 μ M



B. Tomoxetine 1 μ M

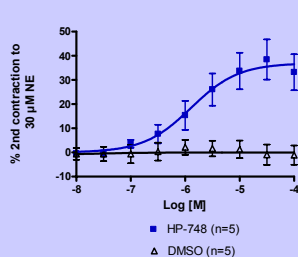


- The solvent for besipirdine and tomoxetine has no effect since the pEC₅₀ value for the 1st CRC to NE was 5.47 (5.25-5.69, 95% C.I.) whereas the corresponding value for the 2nd CRC to NE after solvent incubation was 5.48 (5.30-5.65; 95% C.I.). This difference was not statistically significant (P > 0.05).
- Besipirdine at 1 μ M, shifted to the left the 2nd CRC to NE, the pEC₅₀ values being 5.02 (4.88-5.15; 95 % C.I.) and 5.60 (5.47-5.74, 95% C.I.), before and after incubation (Fig. A).
- Tomoxetine had a similar effect, the pEC₅₀ values being 5.43 (5.28-5.57; 95 % C.I.) and 6.04 (5.90-6.19, 95% C.I.), before and after incubation (Fig. B). These differences were statistically significant (P < 0.0001 for both besipirdine and tomoxetine).

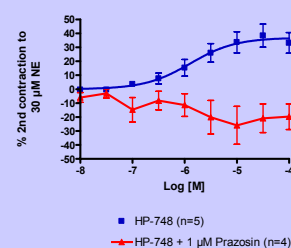
Protocol 2: α_1 -adrenoceptor agonism

Effect of HP748 on the basal tone of the rabbit isolated urethra

A. Effect of HP748 or its solvent



B. Effect of HP748 in the absence and presence of 1 μ M prazosin



- Besipirdine up to 100 μ M was completely devoid of direct contractile activity (data not shown).
- HP748 induced a concentration-dependent contraction of the rabbit isolated urethra, starting from 0.3 μ M and reaching a plateau at 30 μ M. Curve fitting estimated an pEC₅₀ value of 5.89 (5.49-6.30, 95% C.I.) and an Emax value of 37.0% of the 2nd contraction to 30 μ M NE (29.9-44.1%, 95% C.I.).
- Prazosin (1 μ M; 30 min equilibration period) completely blocked the CRC to HP748. These results are illustrated in Figure 2 (A-B).

DISCUSSION

Tomoxetine is a well known NE reuptake inhibitor. It was previously reported that this compound potentialized the CRC to NE in the rabbit isolated urethra (2). In our hands, 1 μ M tomoxetine shifted to the left, in a statistically significant manner, the 2nd CRC to NE, therefore validating our experimental protocol.

The potentiating effect of 1 μ M besipirdine on the 2nd CRC to NE is in accordance with its previously determined potency (IC₅₀ = 13.3 nM) as NE reuptake inhibitor of the recombinant human transporter (UroGene data on file).

On the rabbit isolated urethra HP748 at 30 μ M induced a contraction equal to 37% of the response to 30 μ M NE. Since this contraction was totally abolished following incubation with 1 μ M prazosin, we conclude that HP748 is a partial agonist of the α_{1A}/α_{1L} adrenoceptor subtype, known to be functional in the rabbit isolated urethra (3). This result is in accordance with a previous study reporting that HP748 was a partial agonist on the rat isolated aorta (1), whereas besipirdine, as in our experimental protocol, was ineffective.

CONCLUSIONS

Besipirdine potentializes the CRC to NE in the rabbit isolated urethra, whereas its main metabolite (HP748) is a partial agonist of the α_{1A}/α_{1L} adrenoceptor subtype. This receptor, having a lower affinity for prazosin than the typical α_1 -adrenoceptor subtypes, was also described in the human urethra (4). Considering that adrenergic innervation has an important role in maintaining urethral closure pressure (5), we conclude that besipirdine could be useful to treat stress urinary incontinence in humans.

REFERENCES

- (1) Hubbard JW *et al.* J Pharmacol Exp Ther 281: 337-346, 1997; (2) Foreman MM & McNulty AM. Life Sci. 53: 193-200, 1993; (3) Van der Graaf PH *et al.* Eur. J. Pharmacol. 327: 25-32, 1997; (4) Fukasawa R *et al.* Br. J. Urol. 82: 733-37, 1998; (5) Brading AF *et al.* Eur Urol 36: 74-79, 1999.