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EFFECTS OF BESIPIRDINE AND ITS MAIN METABOLITE ON THE CONTRACTILITY OF THE RABBIT ISOLATED URETHRA

Hypothesis / aims of 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). However, their effects 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.

Study design, materials and methods

Adult female rabbits were killed by cervical dislocation and exsanguinated. Two smooth muscle strips were obtained from medial-distal urethra and mounted in organ baths containing a Krebs solution (pH 7.4, gassed with 95% O_2 and 5% CO_2 at 37°C). Propranolol (1µM), normetanephrine (1 µM) desipramine (0.1 µM) and deoxycorticosterone (3 µM) 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 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 µM), 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 µM was performed. After a new 30 min washout period, besipirdine or tomoxetine (both at 1 µM) were incubated for 30 min, then a second CRC to NE was completed. The results were expressed as pEC_{50} 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 µM, in the absence or presence of 1 µM 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 µM NE.

Figure 1: Effect of besipirdine (A) and tomoxetine (B) on NE-induced contractions on rabbit isolated urethra

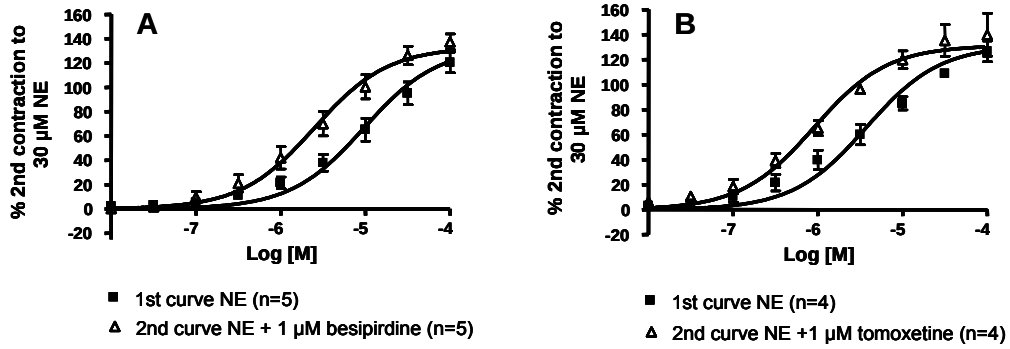
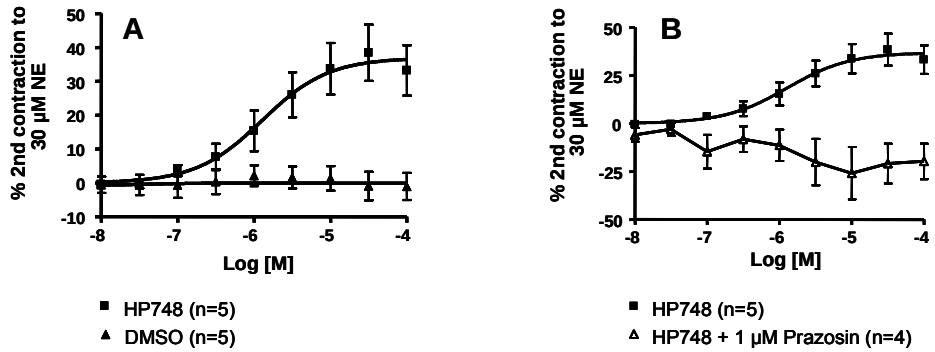


Figure 2: Contractile effect of HP748 or its solvent (A) in the absence and presence of prazosin (B) on rabbit isolated urethra



Results

Protocol 1: Besipirdine at 1 µM, shifted to the left the 2nd CRC to NE, the pEC_{50} 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 with besipirdine (Fig.1A). Tomoxetine had a similar effect, the pEC_{50} 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 with tomoxetine (Fig. 1B). These differences were statistically significant ($P < 0.0001$ for both besipirdine and tomoxetine).

Besipirdine and tomoxetine solvent has no effect since the pEC_{50} 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 ($P > 0.05$).

Protocol 2: HP748 induced a concentration-dependent contraction of the rabbit isolated urethra, starting from 0.3 µM and reaching a plateau at 30 µM. Curve fitting estimated an pEC_{50} value of 5.89 (5.49-6.30, 95% C.I.) and an Emax value of 37.0% of the 2nd contraction to 30 µM NE (29.9-44.1%, 95% C.I.). Prazosin (1 µM; 30 min equilibration period) completely blocked the CRC to HP748. These results are illustrated in Figure 2 (A-B). Besipirdine up to 100 µM was completely devoid of activity (data not shown).

Interpretation of results

On the test for NE reuptake inhibition, besipirdine showed potency similar to that of tomoxetine, a well known NE reuptake inhibitor. The potentiating effect of 1 µM Besipirdine on the 2nd CRC to NE is in accordance with its previously determined potency (IC_{50} = 13.3 nM) as NE reuptake inhibitor of the recombinant human transporter.
On the rabbit isolated urethra HP748 at 30 µM induced a contraction equal to 37% of the response to 30 µM NE. Since this contraction was totally abolished following incubation with 1 µM prazosin, we conclude that HP748 is a partial agonist of the α_{1A}/α_{1L} adrenoceptor subtype expressed in rabbit urethra. 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.

Concluding message

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. Considering that adrenergic innervation has an important role in maintaining urethral closure pressure (2), we conclude that besipirdine could be useful to treat stress urinary incontinence (SUI) in humans.

References

1) J Pharmacol Exp Ther **281**: 337-346, 1997.
2) Eur Urol **36**: 74-79, 1999.

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