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EVALUATION OF THE B3-ADRENOCEPTOR AGONIST, CL-316243, ON DETRUSOR FUNCTION IN THE SPONTANEOUSLY HYPERTENSIVE RAT

Hypothesis / aims of study

The spontaneously hypertensive rat (SHR) exhibits an overactive bladder phenotype and may be a useful animal model of detrusor overactivity. The aetiology of the detrusor hyper-reflexia in this strain is poorly understood, but may be related to increased sympathetic drive to the lower urinary tract (1). In other acute and chronic rodent models of bladder hyperreflexia, the selective β3-adrenoceptor agonist, CL-316243 (2), increases bladder capacity and inhibits non-voiding contractions (3); however, the effects of this agent on detrusor hyper-excitability in the SHR have yet to be reported. The aims of the present study were to evaluate the effects of CL-316243 on bladder and cardiovascular function in the SHR and to compare the concentration-effect relationship on the bladder *in vivo* with that determined in isolated detrusor strips.

Study design, materials and methods

Studies were conducted with female SHRs (190-250 g) in accordance with United Kingdom legislation and subject to local ethical review.

In vitro: Urothelium intact, longitudinal bladder strips were mounted under 10 mN of tension and allowed to equilibrate for 60-90 min in Krebs – Ringer buffer. After washout, the preparations were incubated with 1μM phentolamine and subsequently contracted with 60 mM KCl until a stable contraction developed. CL-316, 243 (0.01nM-10μM) was then added cumulatively to the organ bath and the resulting responses were expressed as a percentage of the relaxation induced by 100μM nifedipine. Concentration-effect curves were then plotted for each strip using an ordinary least squares method (unconstrained parameters). EC_{50} values were determined together with the maximal response for each strip and expressed as respective geometric or arithmetic means with 95% confidence limits for the whole data set.

In vivo: A jugular vein, a carotid artery and the urinary bladder were each cannulated in urethane-anaesthetised rats (1.25g/kg, iv). Saline was continuously infused (45 μl/min) into the bladder to evoke micturition. After a stabilisation period of 1 hour, baseline cystometrograms were recorded for 30 min. Loading and maintenance doses of compound (to target free plasma concentrations of 0.1, 1 and 10 nM) were each given intravenously over consecutive 30 min periods. Blood samples were taken at the end of each dosing period and the resulting plasma was analysed by mass spectrometry to determine the free concentration of CL-316, 243. Vehicle (saline) controls were performed concurrently. Responses were expressed as a percentage of the baseline level and geometric mean or median together with respective 95% confidence limits or lower and upper quartiles were calculated. Comparisons were made using a Mann-Whitney test and P values less than 0.05 were considered indicative of statistical significance.

Results

In vitro: CL-316, 243 produced a concentration-dependent relaxation of KCl-pre-contracted strips of detrusor with an EC_{50} value of 18 nM (11, 28) and mean maximal relaxation of 57% (47, 66) of the nifedipine-induced relaxation (N=12 detrusors, n=19 detrusor strips). *In vivo:* Anaesthetised SHRs exhibited pronounced non-voiding contractions on their cystometrograms, which were dose-dependently inhibited by CL-316, 243. At a targeted concentration of 10 nM, the frequency of these was reduced to 6% (0, 51) of baseline ($P<0.01$ compared with vehicle control). The compound also significantly reduced the peak void pressure at this targeted level to 82% (77, 89) of baseline ($P<0.05$ compared with vehicle control). However, CL-316, 243 had no significant effect upon voiding frequency, heart rate or blood pressure compared with vehicle-treated preparations. Plasma analysis revealed a close correspondence between the targeted and measured free plasma level, which at the 10 nM target concentration was 8 nM (4, 14).

Interpretation of results

The results of the present study demonstrate that CL-316, 243 relaxes detrusor strips from SHR's *in vitro* with a potency comparable to that reported for detrusor strips from wild type rats and indicative of relaxation via stimulation of β3-adrenoceptors (2). Furthermore, given the coherence between the *in vitro* and *in vivo* potency of CL-316, 243, it seems logical to infer that activation of this receptor mechanism at the level of the detrusor mediates inhibition of non-voiding contractions and reduction of peak void pressure in anaesthetised animals. In contrast to studies in other animal models of detrusor overactivity, no effect on voiding frequency was observed with CL-316, 243 and further studies are warranted to investigate this apparent discrepancy.

Concluding message

Analogous to some observations in other rat models of bladder hyperactivity (3), we show that in the SHR, CL-316243 mediates inhibition of non-voiding contractions and reduces peak void pressure. These effects are consistent with the induction of β3-adrenoceptor mediated relaxation of detrusor smooth muscle and occur in the absence of significant cardiovascular effects. Thus, the current study concurs with the view that the SHR exhibits an overactive bladder phenotype and, moreover, adds to the growing body of evidence that selective agonists of β3-adrenoceptors might have clinical utility in the management of detrusor overactivity.

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