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THE EFFECT OF THE UROTHELIUM ON B-ADRENOCEPTOR-MEDIATED RELAXATORY AND INHIBITORY EFFECTS IN PIG BLADDER TRIGONE AND PROXIMAL URETHRA

Hypothesis / aims of study

Recently the function of the bladder urothelium has become a focus of interest, and it is thought that the urothelium releases several neurotransmitters. We have recently shown that β-adrenoceptor agonists have a greater inhibitory effect on detrusor contraction in bladder dome when the urothelium is intact and this is mediated via the β_3-adrenoceptor subtype [1]. However, little information exists regarding the role of the urothelium in the bladder trigone and proximal urethra. Therefore, we investigated whether the urothelium can modify relaxatory and inhibitory responses to β-adrenoceptor agonists in the bladder trigone and proximal urethra, and which β-adrenoceptor subtypes are involved.

Study design, materials and methods

Paired longitudinal strips of bladder trigone and proximal urethra were isolated from fresh pig bladders. The urothelium was removed from 1 strip per pair and the strips mounted in tissue baths in gassed Krebs solution at 37°C. Relaxation experiments were performed by cumulative addition of β-adrenoceptor agonists (isoprenaline, dobutamine, salbutamol, or BRL37344; non-selective, β_1, β_2 and β_3 selective respectively) to tissues pre-contracted with carbachol. In separate experiments the relaxing effects of isoprenaline were studied following incubation with several inhibitors or antagonists (atropine (10 μM), suramin (100 μM), or indomethacin (10 μM); muscarinic receptor antagonist, P2 receptor antagonist, or COX inhibitor respectively). Inhibition experiments were performed by obtaining carbachol-response curves in the presence and absence of β-adrenoceptor agonists (10 μM) (dobutamine, salbutamol, or BRL37344). In separate experiments the inhibitory effects of isoprenaline (10 μM) were studied following incubation with 1μM of a β-adrenoceptor antagonist (propranolol, CGP20712, ICI-118, 551, or SRL59230A; non selective, β_1, β_2 and β_3 selective respectively). Results are mean ± SEM, and a paired or non-paired Student's t-test was used for statistical analysis.

Results

Isoprenaline, salbutamol, and BRL37344 relaxed carbachol-precontracted tissues respectively. However, dobutamine did not produce any significant relaxation. The potencies (pEC_{50}) of isoprenaline and salbutamol in relaxing the tissues were significantly increased in the absence of the urothelium versus the presence (isoprenaline: trigone 7.18±0.18 vs 6.64±0.15 $p<0.01$ (n=9); proximal urethra 6.93±0.12 vs 6.40±0.11 $p=0.032$ (n=6); salbutamol: trigone 6.43±0.16 vs 5.87±0.14 $p=0.035$ (n=5); proximal urethra 6.16±0.13 vs 5.55±0.21 $p=0.017$ (n=6)). However these urothelial-dependent differences were not observed with dobutamine or BRL37344. In contrast, maximum relaxations to isoprenaline, salbutamol or BRL37344 were similar in the presence and absence of the urothelium. Relaxations mediated by isoprenaline were not affected by atropine, suramin or indomethacin.
Contractile responses to carbachol were depressed by 28.7±5.6 % (trigone) and 22.1±5.3 % (proximal urethra) in the presence of the urothelium. The potencies of carbachol were also significantly reduced ($p<0.05$) in the presence versus the absence of the urothelium (trigone 5.34±0.09 vs 5.61±0.09 (n=51); proximal urethra 5.53±0.08 vs 5.76±0.09 (n=49)). β-adrenoceptor agonists caused rightward shifts of carbachol-response curves. There was a greater shift with BRL37344 in the presence versus the absence of the urothelium. However this was not observed with dobutamine or salbutamol (Table 1). The non-selective β-adrenoceptor agonist isoprenaline produced a greater shift in the presence of the urothelium than in the absence, and this was still observed in tissues pre-incubated with propranolol, CGP20712 or ICI-118, 551. However, this difference was not observed when tissues were pre-incubated with SRL59230A (Table 1).

Interpretation of results

In relaxation experiments the potencies of isoprenaline and salbutamol in relaxing trigone and proximal urethra were significantly increased in the absence of the urothelium, although this was not observed with dobutamine or BRL37344. These results suggest that β_2-adrenoceptor agonists may stimulate the release of a substance from the urothelium/suburothelium, which acts against relaxation. In inhibitory experiments the β_3-adrenoceptor selective agent BRL37344 was the only selective agonist to have a greater inhibitory effect on detrusor contraction when the urothelium was intact. This suggests that β_3 -adrenoceptors on the urothelium may mediate these inhibitory effects. This is supported by the effect being antagonised only by the selective β_3-adrenoceptor antagonist SRL59230A.

Concluding message

In pig bladder trigone and proximal urethra, these data suggest that β_2-selective adrenoceptor agonists may inhibit detrusor relaxations via an action at the urothelium. In contrast β_3-selective adrenoceptor agonists acting via the urothelium can inhibit detrusor contractions.

References

[1] Eur Urol (2007) 6; Suppl 40

Table 1. The comparison of potencies and mean shifts in intact and denuded strips

	Drug	Urothelium	Control (pEC50)	Drug (pEC50)	Mean shift
Trigone	Dobutamine	Intact	5.32±0.04	5.30±0.14	1.18±0.13
		Denuded	5.63±0.03	5.61±0.12	1.14±0.17
	Salbutamol	Intact	5.24±0.03	4.90±0.12	2.26±0.28

		Denuded	5.41±0.06	5.08±0.10	2.28±0.46
	BRL37344	Intact	5.36±0.02	4.91±0.07	2.95±0.27*
		Denuded	5.65±0.06	5.46±0.24	1.83±0.36
	Isoprenaline	Intact	5.13±0.08	4.65±0.31	4.18±0.47*
		Denuded	5.47±0.06	5.21±0.24	2.71±0.40
	Isoprenaline	Intact	5.30±0.04	4.88±0.13	2.59±0.36*
	+propranorol	Denuded	5.64±0.08	5.48±0.18	1.26±0.14
	Isoprenaline	Intact	5.32±0.04	4.66±0.10	4.13±0.51*
	+CGP20712	Denuded	5.65±0.04	5.20±0.18	2.59±0.39
	Isoprenaline	Intact	5.27±0.05	4.84±0.12	2.84±0.12*
	+ICI-118, 551	Denuded	5.70±0.04	5.57±0.14	1.49±0.39
	Isoprenaline	Intact	5.39±0.05	5.11±0.13	1.78±0.28
	+SRL59230A	Denuded	5.53±0.04	5.28±0.20	1.70±0.27
Proximal	Dobutamine	Intact	5.42±0.04	5.33±0.13	1.33±0.21
Urethra		Denuded	5.79±0.05	5.64±0.12	1.30±0.11
	Salbutamol	Intact	5.68±0.03	5.39±0.13	1.97±0.24
		Denuded	5.81±0.03	5.55±0.06	2.01±0.32
	BRL37344	Intact	5.33±0.07	4.96±0.14	3.02±0.24*
		Denuded	5.54±0.06	5.30±0.11	1.98±0.33
	Isoprenaline	Intact	5.35±0.06	4.67±0.13	4.09±0.54*
		Denuded	5.71±0.04	5.33±0.08	2.46±0.37
	Isoprenaline	Intact	5.62±0.05	5.17±0.05	2.80±0.40*
	+propranorol	Denuded	5.84±0.05	5.63±0.11	1.66±0.19
	Isoprenaline	Intact	5.61±0.06	5.08±0.11	3.60±0.41*
	+CGP20712	Denuded	5.85±0.05	5.56±0.18	2.31±0.30
	Isoprenaline	Intact	5.55±0.05	5.11±0.09	3.16±0.47*
	+ICI-118, 551	Denuded	5.82±0.05	5.50±0.14	1.90±0.27
	Isoprenaline	Intact	5.53±0.04	5.20±0.18	1.73±0.11
	+SRL59230A	Denuded	5.72±0.05	5.45±0.09	1.80±0.29

Values represent means ± S.E.M. of the results
*Significantly different from denuded strips ($p<0.05$)

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**No**
Statement that no ethical approval was needed	**We got pig bladders from the abbatoir in Sheffield, UK. No ethical approval was needed.**