

## EFFECTS OF FASUDIL, A RHO-KINASE INHIBITOR, FOR CONTRACTION OF THE PIG BLADDER TISSUES WITH OR WITHOUT UROTHELIUM

### Hypothesis / aims of study

The Rho/ Rho-associated serine-threonine protein kinase (ROCK) pathway plays an important role in regulating cell morphology and migration by reorganizing the actin cytoskeleton as well as vascular smooth muscle contraction. In addition, this pathway has been reported to take part in the contraction of non-vascular smooth muscle at such sites as the rabbit bladder by modifying the sensitivity of contractile and regulatory proteins to  $[Ca^{2+}]_i$ , the phenomenon referred to as "Ca<sup>2+</sup> sensitization". We investigated the effects of fasudil, a ROCK inhibitor, on the contraction of the pig urinary bladder tissues with or without urothelium.

### Study design, materials and methods

Detrusor muscle and urothelium from the dome of the pig bladder were obtained. The cumulative concentration-response curves (CRCs) to carbachol were obtained both with and without 3-10  $\mu$ M fasudil, a ROCK inhibitor, and compared the effects of the drug on carbachol-induced contraction of pig urinary bladder smooth muscle between those with or without urothelium. The inhibitory responses of fasudil (10nM-100 $\mu$ M) were also examined in tissues precontracted with KCl and carbachol, and the responses to field electric stimulation in the pig bladder with and without urothelium.

### Results

In muscle strips without mucosa (n=24), Emax decreased significantly after administration at concentrations of 3 and 10 $\mu$ M (both  $p < 0.01$ ), but did not change after the administration of 30 $\mu$ M fasudil, and the changes were not concentration-dependent (72.5 $\pm$ 7.43%, 58.4 $\pm$ 8.04% and 68.36 $\pm$ 9.6%, of respectively, of the first curve). pEC<sub>50</sub> after the administration of 3 and 10  $\mu$ M fasudil (5.05 $\pm$ 0.09 and 5.08 $\pm$ 0.12, respectively) was significantly less than that for the first curve ( $p < 0.001$  each), but it did not change significantly after administration of 30 $\mu$ M fasudil (5.15 $\pm$ 0.14). In muscle strips with an intact mucosa (n=12), Emax significantly decreased (all  $P < 0.05$ ) after the addition of 3, 10 and 30  $\mu$ M fasudil to 84.9 $\pm$ 6.7%, 67.9 $\pm$ 5.2% and 35.2 $\pm$ 4.1%, respectively, of that obtained for the first curve. pEC<sub>50</sub> also decreased significantly after the addition of 3 and 10 $\mu$ M fasudil (5.00 $\pm$ 0.07 and 4.60 $\pm$ 0.08, respectively;  $p < 0.01$  and  $p < 0.01$ , respectively), but it did not change significantly after the administration of 30  $\mu$ M fasudil (5.17 $\pm$ 0.11). In tissues precontracted with 80mM KCl, tension after the administration of fasudil decreased by 40.8 $\pm$ 6.8% and 40.9 $\pm$ 6.8% from the baseline value in muscle strips without urothelium (n=6) and with urothelium (n=6), respectively. Tension tended to decrease after administration of fasudil compared to the control value in muscle strips without urothelium ( $P = 0.0614$ ) and with urothelium ( $p = 0.0603$ ). In tissues precontracted with 100 $\mu$ M carbachol, tension after the administration of fasudil (1nM-100 $\mu$ M) was significantly decreased by 41.5 $\pm$ 4.2% and 42.9 $\pm$ 4.9%, respectively, from the baseline value obtained after pre-contraction with carbachol, for muscle strips without urothelium (n=12) or with urothelium (n=5) (both  $p < 0.05$ ). The responses to electrical field stimulation at 1 Hz, 5 Hz, 10 Hz, 20Hz and 50Hz were significantly decreased after addition of fasudil (3, 10, and 30  $\mu$ M), in a concentration-dependent manner in both tissues without and with urothelium.

### Interpretation of results

The relaxation response to ROCK inhibitors may be not only due to direct effects on detrusor, but also due to Rho/ROCK pathway from the urothelium.

### Concluding message

Fasudil showed a stronger inhibitory effect on CRC to carbachol in tissue preparations with an intact urothelium than in those without urothelium. Fasudil also had an inhibitory effect on carbachol-induced contraction at high concentrations (higher than 10 $\mu$ M) and on contraction produced by electrical field stimulation in both tissues with and without urothelium.

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<b>What were the subjects in the study?</b>	<b>ANIMAL</b>
<b>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</b>	<b>No</b>
<b>Statement that no ethical approval was needed</b>	<b>No animal ethical committee approval was required because the tissues were obtained from an abattoir.</b>