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EFFECTS OF N-ARACHIDONOYL-(2-METHYL-4-HYDROXYPHENYL) AMINE (VDM11), AN ANANDAMIDE TRANSPORTER INHIBITOR, ON THE MICTURITION REFLEX IN URETHANE-ANESTHETIZED RATS

Hypothesis / aims of study
Anandamide, an endogenous cannabinoid (CB) receptor ligand, is an ethanolamine amide of arachidonic acid that was first isolated from porcine brain (1). Anandamide is reportedly synthesized in macrophages, vascular endothelium, and primary afferent fibers (2). Recent studies have also demonstrated that an anandamide plays an important role in the modulation of micturition reflex (3). However, it is unknown whether the anandamide membrane transporter plays a role in this modulation. The aim of this study is to investigate the effects of N-arachidonoyl-(2-methyl-4-hydroxyphenyl) amine (VDM11), an anandamide membrane transporter inhibitor, on the micturition reflex in rats.

Study design, materials and methods
Adult female Sprague-Dawley rats weighing 247 to 261 g were used. Rats were anesthetized with isoflurane followed by urethane (1.2 g/kg subcutaneously). Thereafter the abdomen was opened through a midline incision and a PE-60 polyethylene catheter was implanted into the bladder through the bladder dome. The catheter was connected with a three-way stopcock to a pressure transducer and a pump for continuous saline infusion (0.04 ml/min). A PE-10 catheter was also inserted into the right jugular vein for intravenous injections. The effects of intravenous administration of VDM11, with or without CB receptor antagonists, on the cystometric parameters were evaluated. Saline was continuously infused for 2 hours to evaluate bladder activity during a control period. VDM11 (1, 3 and 10 mg/kg, n=6 per dose) was then injected intravenously to evaluate changes in bladder activity. In experiments examining the effects of CB receptor antagonists, VDM11 (10 mg/kg) was injected intravenously when the first bladder contraction was observed after intravenous administration of AM251, a CB1 antagonist (3 mg/kg, n=6), or AM630, a CB2 antagonist (3 mg/kg, n=6). All data values are expressed as the mean ± SE. Statistical significance was determined with one-way ANOVA with $p<0.05$ considered significant.

Results
Intravenous administration of VDM11 at 1, 3 and 10 mg/kg increased intercontraction intervals at doses of 3 mg/kg or higher in dose dependent fashion to 103.3 ± 2.3%, 117.2 ± 3.6% and 144.5 ± 10.6% of the control value, respectively (at 3 and 10 mg/kg, $p<0.01$). These inhibitory effects were observed immediately after administration. Intravenous administration of VDM11 at 1, 3 and 10 mg/kg also increased threshold pressure at doses of 3 mg/kg or higher in dose dependent fashion to 100.7 ± 2.9%, 138.9 ± 22.3% and 183.4 ± 29.1% of the control value, respectively (at 3 and 10 mg/kg, $p<0.01$). There were no significant changes in basal pressure or maximum pressure at any doses tested. When AM251 was administered one voiding cycle before VDM11 administration, the increases in intercontraction intervals and threshold pressure induced by VDM11 administration alone were not seen. In contrast, when AM630 was administered before VDM11 administration, increases in intercontraction intervals and threshold pressure (141.8 ± 10.9% and 169.9 ± 14.6% of the control value, respectively) were observed, as they were after VDM11 alone.

Interpretation of results
In urethane-anesthetized rats, suppression of anandamide transporters by VDM11 has an inhibitory effect on the micturition reflex, as evidenced by the increases in intercontraction intervals and threshold pressure. The main function of VDM11 seems to be mediated by modulation of afferent activity, rather than efferent or smooth muscle activity, because VDM11 induced increases in intercontraction intervals and threshold pressure without affecting maximum pressure or basal pressure. When AM251, but not AM630, was administered before VDM11, the VDM11-induced increases in the intercontraction intervals and threshold pressure were prevented, indicating that VDM11-induced increases in intercontraction intervals and threshold pressure were mediated by activation of CB1 receptors.

Concluding message
These results suggest that anandamide, an endogenous CB ligand, may modulate the micturition reflex and that anandamide transporters play an important role in this modulation. Furthermore, these findings indicate that in urethane-anesthetized rats inhibition of the uptake of anandamide can inhibit the micturition reflex and these inhibitory effects of VDM11 are at least in part mediated by the CB1 receptor. Thus the anandamide transporter could be a potential target for the treatment of bladder dysfunction such as overactive bladder.

References
1. Science (1992) 258; 1946-1949.
2. J Neurochem (2003) 84; 1-7.
3. Urology (2007) 70; 202-208.

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