Studies on Mode of Inhibitory Effects of Tetramethylpyrazine and Ferulic Acid on Spontaneous Movement of Rat Uterus in situ

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We reported that tetramethylpyrazine and ferulic acid inhibited spontaneous uterine contractions in rats in situ and the inhibitory effect induced by the combination of the two compounds was potentialize effect. The present work was carried out to study the mode of the inhibitory action of tetramethylpyrazine and ferulic acid on spontaneous uterine contractions in rats in situ. The inhibitory effect induced by tetramethylpyrazine was blocked by propranolol, but not by cimetidine, and the inhibitory effect was produced also in reserpinized rats. Tetramethylpyrazine slightly inhibited the 5-hydroxytryptamine- or oxytocin-induced uterine contractions but scarcely the acetylcholine-induced contraction. The inhibitory effect induced by ferulic acid was blocked neither by propranolol nor by cimetidine. Ferulic acid inhibited the oxytocin-induced uterine contraction strongly, but not the acetylcholine- or 5-hydroxytryptamine-induced contraction. Indomethacin did not affect the inhibitory effects induced by tetramethylpyrazine and ferulic acid. The followings were suggested: 1; tetramethylpyrazine mainly acts on the β-adrenoceptor directly and ferulic acid partly on the oxytocin-receptor system, 2; neither of the inhibitory effects induced by the two compounds is due to their inhibitory action on the prostaglandin biosynthesis, 3; since the active sites of the two compounds are different, the effect on rat uterus induced by a combination of tetramethylpyrazine and ferulic acid may be potentialize effect.

Keywords—tetramethylpyrazine; ferulic acid; uterine contraction; inhibition; β-adrenoceptor; oxytocin-receptor

Tetramethylpyrazine (TMP) is an alkaloid contained in Ligusticum wallichii FRANCH and ferulic acid (FA) is a phenolic compound contained in Ligusticum wallichii FRANCH and Angelica sinensis (OLIV.) DIELS. Ligusticum wallichii and Angelica sinensis are compatible and they have been frequently used in combination in traditional Chinese medicine, especially as a homeostatic remedy for women's disorders and menoxenia, as well as an analgesic for dysmenorrhea, etc.

Previously, we reported that TMP and FA inhibited spontaneous uterine contractions, and that when used in combination, they synergically inhibited the contractions of rat uterus in situ. It was also reported that TMP inhibited the tension of platelet preparation and the contraction of isolated heart, uterus and vascular smooth muscle preparations in vitro. The mechanism of inhibition by TMP has not been known clearly yet and there are few reports on the mechanism of inhibition of the uterine smooth muscle contraction by FA.

The present study was carried out to elucidate the mode of the TMP- and FA-induced inhibitory actions on spontaneous rat uterine contractions in situ.

Materials and Methods

Tetramethylpyrazine (TMP) (Beijin Institute of Pharmaceutical Industry of China) was dissolved in 0.9% saline solution. Ferulic acid (FA) (Shanghai First Reagent Factory of China) was dissolved in 0.9% saline solution.
containing an equivalent amount of sodium hydroxide to neutralize the acid.

Oxytocin (Oxy, Teikokuzoki), acetylcholine chloride (Ach, Daichii), cimetidine (Cime, Sigma), 5-hydroxytryptamine (5-HT, Sigma), isoproterenol hydrochloride (Iso, Sigma), propranolol hydrochloride (Prop, Sigma), indomethacin (Sigma), cyproheptadine hydrochloride (Sigma), histamine dihydrochloride (Hist, Wako), reserpine (Wako), atropine sulfate (Wako), papaverine hydrochloride (Wako) and tyramine hydrochloride (Wako) were dissolved in 0.9% saline solution.

Methods
Adult virgin female Wistar rats weighing 180-230 g were used. Only those rats in estrus of the estrous cycle were used: their estrous stages were examined by the vaginal smear tests in the morning of the day of experiment. The rats were anesthetized with urethane (1.5 g/kg, i.p.). Tracheotomy was performed. One jugular vein was cannulated for the i.v. administration of the test solution.

The uterine horns were exposed at the cervical junction by a low abdominal incision over the bladder area. One horn was lifted up gently and gripped with a small clip 1 cm away from the cervical junction. The clip was connected to a force-displacement transducer, with which the uterine contractile movements were recorded isometrically and integrated (EI-601G, Nihon Koden) on a polygraph (RM-6200, Nihon Koden) connected. In each case, the initial load was 1 g.

Reserpineized rats were prepared by giving reserpine at 2 mg/kg s.c. for three days before the test.6) The test compounds, except reserpine, were injected intravenously.

Statistical analysis
In the case of Oxy, Ach and 5-HT, the uterine contraction induced by Oxy, Ach or 5-HT was taken as 100%, and the inhibitory effects produced by TMP and FA on the Oxy-, Ach- or 5-HT-induced contraction were expressed in terms of percentages of the uterine responses to a single injection of Oxy, Ach or 5-HT. In the case of Hist and Iso, the average integral value during the 5 min before the administration of a test solution was taken as 100% and the effects of the test compound were expressed as percentages of the average integral value of contraction before TMP and FA administration.

The ID_{50} value was obtained by Finney's probit analysis.

Results

Effect of TMP and FA on oxy-induced uterine contractions
Administration of Oxy, at 0.3 unit/kg, immediately produced a strong contraction of rat uterus and the duration was about 10 min. When TMP was administered singly, at 30, 100 and 200 mg/kg, or FA, at 50, 100 and 300 mg/kg, TMP and FA produced a dose-dependent inhibitory effect on the Oxy-induced contractions. TMP, at 200 mg/kg, or FA, at 300 mg/kg, inhibited the Oxy-induced contractions completely, whereas, TMP, at 10 mg/kg, or FA, at 30 mg/kg, did not inhibit the Oxy-induced contractions at all. Papaverine, at 1, 3 and 10 mg/kg, also produced a dose-dependent inhibitory effect. The results are given in Fig. 1 and their ID_{50} in Table I.

Effect of TMP and FA on ach-induced uterine contraction
Administration of Ach, at 0.1 mg/kg, produced a single strong contraction and the duration was about 2 min. The contraction was inhibited by 200 mg/kg of TMP by 35% and by 300 mg/kg of FA by 23%. Papaverine, at 1, 3 and 10 mg/kg, produced a dose-dependent inhibitory effect and at 10 mg/kg, the inhibition was 92%. Atropine, at 30 μg/kg, inhibited the Ach-induced contraction completely. The results are given in Fig. 2 and their ID_{50} in Table I.

Effect of TMP and FA on 5-HT-induced uterine contraction
Administration of 5-HT at 10 μg/kg produced a single strong contraction and the duration was about 2 min. A dose-dependent inhibitory effect was produced by 30, 50 and 100 mg/kg of TMP on 5-HT-induced contraction. The inhibition by 100 mg/kg of TMP was 92%, whereas that by FA at 300 mg/kg was only 45%. Papaverine produced a dose-dependent inhibitory effect on 5-HT-induced contraction at doses of 1, 3 and 10 mg/kg and the inhibition by 10 mg/kg of papaverine was about 85%. Cyproheptadine inhibited the 5-HT-induced contraction by 78%, at 1 mg/kg. The results are given in Fig. 3 and their ID_{50} in Table I.

Effect of TMP and FA on uterine contractions of cimetidine-treated rats
TMP, at 50 mg/kg, FA, at 100 mg/kg and Hist, at 0.1 mg/kg, decreased the frequency, the amplitude and the tone of spontaneous rat uterine contractions. When the rats had been pretreated with 3 mg/kg of Cime, an inhibitory effect was produced by TMP and FA, but not by Hist. However, the blocking of the effect of Hist by Cime gradually disappeared during the following 60 min. The results are given
Fig. 1. Antagonistic Effect of Tetramethylpyrazine, Ferulic Acid and Papaverine on Oxytocin-induced Contraction in Rat Uterus

TABLE I. ID_{50} Values of Tetramethylpyrazine, Ferulic Acid and Papaverine on Oxytocin, 5-Hydroxytryptamine and Acetylcholine-induced Contraction and Spontaneous Contraction in Rat Uterus

<table>
<thead>
<tr>
<th>Compounds</th>
<th>ID_{50} on Uterine Contraction (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxy-induced</td>
</tr>
<tr>
<td>Tetramethylpyrazine</td>
<td>55.0 (26.4-114.7)</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>75.0 (30.8-183.1)</td>
</tr>
<tr>
<td>Papaverine</td>
<td>4.6 (1.2-17.6)</td>
</tr>
</tbody>
</table>


in Fig. 4.

Effect of TMP and FA on uterine contractions of propranolol-treated rats
Like TMP, FA and papaverine, Iso, at 3 \( \mu g/kg \), inhibited the spontaneous rat uterine contractions. The inhibitory effect of Iso, at 3 \( \mu g/kg \), was stronger than that of TMP, at 50 mg/kg, or of FA, at 100 mg/kg. Prop, at 3 mg/kg, increased the frequency, the amplitude and the tone of uterine contractions. On the rats pretreated with Prop, FA showed an inhibitory effect, but TMP and Iso did not: their effects were blocked completely by the pretreatment though the blocking by Prop gradually disappeared during the following 120 min. The results are given in Fig. 5.

Effect of TMP on uterine contractions of reserpinized rats
In the reserpinized rats, TMP, at 30 mg/kg, papaverine, at 3 mg/kg, and Iso, at 3 \( \mu g/kg \), inhibited the spontaneous uterine contractions as in normal rats. Tyramine, at 10 mg/kg, produced an inhibitory effect on spontaneous uterine contractions in normal rats, but not in the reserpinized rat. The results are given in Fig. 6.

Effect of TMP and FA on uterine contractions of indomethacin-treated rats
Pretreatment with indomethacin, at 3 mg/kg, did not affect the spontaneous uterine contractions and did not block the Oxy-induced uterine contractions. The pretreatment did not affect the TMP- and FA-induced inhibitory effect, either.
At the arrow, test compound was intravenously administered.

Ach: acetylcholine, TMP: tetramethylpyrazine, FA: ferulic acid.

**Fig. 2.** Antagonistic Effect of Tetramethylpyrazine, Ferulic acid, Papaverine and Atropine on Acetylcholine-induced Contraction in Rat Uterus

At the arrow, test compound was intravenously.

5-HT: 5-hydroxytryptamine, TMP: tetramethylpyrazine, FA: ferulic acid.

**Fig. 3.** Antagonistic Effect of Tetramethylpyrazine, Ferulic acid, Papaverine and Cyproheptadine on 5-Hydroxytryptamine-induced Contraction in Rat Uterus

**Discussion**

Uterine smooth muscle is characterized by a high degree of spontaneous electrical and contractile activity, which are affected by neurogenic (parasympathetic and sympathetic), hormonal (Oxy, estrogen, etc.) and autacoid (5-HT, Hist, prostaglandins, etc.), and the coordination of which keeps the normal uterine condition. The present study was carried out to elucidate the mode of TMP- and FA-induced inhibitory action on spontaneous rat uterine contractions.
Antagonistic Effect of Cimetidine on Tetramethylpyrazine, Ferulic acid and Histamine-induced Inhibition in Rat Uterus

Fig. 4. Antagonistic Effect of Cimetidine on Tetramethylpyrazine, Ferulic acid and Histamine-induced Inhibition in Rat Uterus

Antagonistic Effect of Propranolol on Tetramethylpyrazine, Ferulic Acid and Isoproterenol-induced Inhibition in Rat Uterus

Fig. 5. Antagonistic Effect of Propranolol on Tetramethylpyrazine, Ferulic Acid and Isoproterenol-induced Inhibition in Rat Uterus

TMP, at 50 mg/kg, strongly inhibited the amplitude, frequency and tone of spontaneous uterine contractions. The inhibition was blocked completely by Prop, but not by Cime. The result suggested that TMP may act on the β-adrenoceptor, but not on the histamine H2-receptor in uterus. It agrees with the facts previously reported that TMP showed a β-adrenoceptor stimulating effect in vivo, increasing the left ventricular pressure, coronary blood flow and heart rate and lowering the arterial blood pressure. TMP inhibited the Oxy- and 5-HT-induced uterine contractions dose-dependently, but the potency was weaker than that of papaverine. Higher doses of TMP did not strongly inhibit the Ach-induced uterine contraction. The ID₅₀ of papaverine for the inhibition of the Oxy-, Ach- and 5-HT-induced contraction was about the same as the ID₅₀ for the inhibition of spontaneous contractions. However, the ID₅₀s of TMP for the inhibition of the Oxy- and 5-HT-induced contractions were about 3 times and 2.4 times that for the inhibition of spontaneous contractions, respectively. These results indicate that
Fig. 6. Inhibitory Effect of Tetramethylpyrazine and Tyramine on Spontaneous Contraction in Reserpinized Rat Uterus

The mode of the inhibitory action of TMP is different from that of papaverine. A $\beta$-adrenoceptor agonist activates adenylyl cyclase and increases cyclic adenosine monophosphate (cyclic AMP) in cells. Cyclic AMP intracellularly inhibits myosinase, blocks the release of calcium from internal stores,$^9$ depresses the arachidonic acid metabolism and produces relaxation of smooth muscles.$^{10,11}$ Wu et al. reported that TMP produced a vasodilation on isolated rat aoruta due to the elevation of cyclic AMP in smooth muscle and that the effect of TMP was similar to that of theophylline, an inhibitor of phosphodiesterase.$^5$

Kalkman reported that the $\beta$-adrenoceptor-mediated function interferes with the 5-HT receptor agonists-induced effect.$^{12}$ So it remains to be elucidated if the inhibitory effect of TMP on the Oxy- and 5-HT-induced contraction is caused by its acting on the $\beta$-adrenoceptor or by direct blocking of the Oxy and 5-HT receptors.

There are $\beta_2$- (inhibitory) and $\alpha_1$- (excitatory) adrenergic receptors in the myometrium of mammals. But almost all sympathetic receptors are $\beta$-adrenergic receptors in non-pregnant rat uterus.$^{13,14}$ In this experiment, rat uterus did not respond to 10 $\mu$g/kg of norepinephrine. Tyramine produced a slight inhibitory effect on uterine contractions and by the administration of prop, the spontaneous uterine contraction was accelerated. This indicates that there is scarcely any $\alpha$-adrenoceptor in the myometrium of non-pregnant rat uterus and therefore, $\beta$-adrenoceptors are main causatives of relaxing uterus.

TMP is considered to directly act on the $\beta$-adrenoceptors in myometrium and the inhibitory effect induced by TMP may be due to the intracellular accumulation of cyclic AMP, because TMP produced an inhibitory effect on the spontaneous uterine contraction of reserpinezed rat, whereas tyramine did not.

FA, at 100 mg/kg, strongly inhibited the spontaneous uterine contractions, especially in amplitude and frequency.$^3$ The inhibition was blocked neither by Cime nor by Prop. On the other hand, FA, at 300 mg/kg, weakly inhibited the Ach- and 5-HT-induced uterine contraction, and strongly the Oxy-induced uterine contractions. The ID$_{50}$ was 1.5 times that for the inhibition of spontaneous uterine contractions. This result suggested that FA might partly act on the oxy-receptor.$^7$

Administration of PGF$_{2\alpha}$ causes strong uterine contractions. It was reported that FA reduced malondialdehyde in platelets,$^{15}$ and that both TMP and FA inhibited the biosynthesis of thromboxane A$_2$. Since indomethacin, which inhibits the biosynthesis of prostaglandins did not affect the TMP- and FA-induced inhibitory effect on spontaneous uterine contractions, the inhibitory effect induced by TMP or FA may not be considered to be related with the biosynthesis of prostaglandins.

From these results, it may be said that TMP mainly acts on the $\beta$-adrenoceptor and FA partly acts on the Oxy-receptor, and that the mode of their inhibitory actions is different from that of papaverine.

As the acting sites of TMP and FA are different, the inhibitory effect induced by a combination of

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TMP with FA on uterus is potentize effect.

References and Notes