Effects of Pollen-Extract Components, Diamines and Derivatives of Feruloylputrescine on Isolated Bladder and Urethral Smooth Muscles of Mice

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Abstract—The contracting or inhibitory effects of pollen-extract components, diamines and derivatives of feruloylputrescine (FP) were investigated on the isolated bladder or urethral smooth muscles of mice. Among the nine diamines ($NH_2 \cdot (CH_2)_n \cdot NH_2$, n=2–10) tested, five of them with shorter carbon chains (n=2–6) (0.1–30.0 mM) only slightly contracted the bladder strips and to some extent inhibited the noradrenaline (NA, 1.77 μM)-induced contraction of urethral strips. 1,5-Diaminopentane (C5), a component of the pollen-extract, inhibited most effectively the NA-induced contraction of urethral strips with an IC50 value of 2.3 mM (95% confidence limit: 2.0–2.6 mM). FP, also a component of the pollen-extract, inhibited the NA-induced contraction of urethral strips in a non-competitive manner, producing 32.5±5.5% (N=5) inhibition at 378 μM. Among the derivatives of FP, feruloylcadaverine inhibited urethral contraction most potently, producing 46.3±7.1% (N=5) inhibition at 359 μM. These derivatives had no effect on bladder contraction. In contrast, four diamines with longer carbon chains (n=7–10) contracted the bladder strips (3–30 mM) and potentiated the NA-induced contraction of urethral strips (10 μM–3 mM). Thus, the components of the pollen-extract, FP and C5, potently inhibited urethral contraction, which may facilitate the discharge of urine in vivo.

Polyamines are known to occur in natural products in high concentrations (1). The pharmacological effects of polyamines, especially spermine and spermidine, on many biological tissues have been reported. The inhibitory or relaxing effects of spermine and spermidine have already been reported in the smooth muscle of the gut (2, 3), uterus (4, 5), respiratory tract (6–8) and vasculature (6). These effects have been explained on the basis of antagonism to calcium ions (4, 8).

In the previous papers, we have reported that T-60, a fraction of the pollen-extract, causes contraction of the bladder and inhibition of urethral contraction, suggesting that it facilitates the discharge of urine in vivo (9). Thereafter, we have found that a water-soluble extract of pollen (T-60) contains putrescine (1,4-diaminobutane: C4), cadaverine (1,5-diaminopentane: C5) and feruloylputrescine (FP) (10). Employing sequential fractionation, we have also found that FP is the active (inhibitory) compound in T-60 (10).

In this study, we investigated the effects of components of pollen-extract, two diamines (C4 and C5), FP and derivatives of FP using bladder and urethral strips isolated from mice. The structure-activity relationship of nine diamines ($NH_2(CH_2)_nNH_2$, n=2–10), including C4 and C5, was also studied.

Materials and Methods

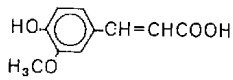
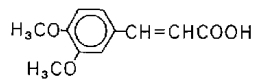
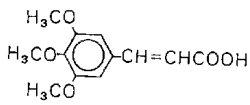
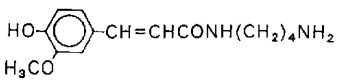
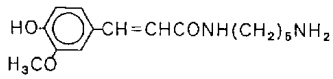
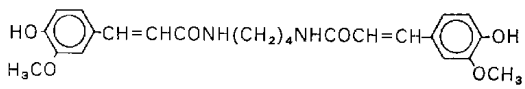
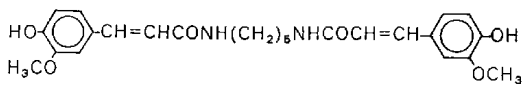
Male ddY mice, weighing 29–47 g, were decapitated and exanguinated. The urethra and the bladder were isolated and cut into muscle strips as previously reported (9).

A modified Krebs solution of the following composition was used: 122 mM NaCl, 5.9 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 15.5

mM $NaHCO_3$ and 11.5 mM glucose. The urethral or bladder strips was suspended in an organ bath containing 5 ml of Krebs solution at 37°C. Resting tension was adjusted to 100 mg in the case of the urethra and 150 mg for the bladder. Isometric tensions were recorded using a Pen-oscillograph (San-ei 8S61-1) with a U-gauge transducer (Shinkoh Tsushin UL-2-240) coupled with a Biophysiograph (San-ei 1236) and a DC amplifier (San-ei 615).

Chemicals used were dl-noradrenaline (NA, Sankyo) and acetylcholine chloride (ACh, Daiichi). Diamines (dihydrochlorides) used were 1,2-diaminoethane (C2), 1,3-diaminopropane (C3), 1,4-diaminobutane

Table 1. The chemical structures of the constituents isolated and identified from pollen-extract (constituents in pollen) and their related compounds including diamines, hydroxycinnamic acid derivatives and hydroxy cinnamic acid amide derivatives

Components in pollen-extract	Related compounds
1,4-Diaminobutane (Putrescine) 1,5-Diaminopentane (Cadaverine) $H_2N(CH_2)_nNH_2$	1,2-Diaminoethane 1,3-Diaminopropane 1,6-Diaminohexane 1,7-Diaminoheptane 1,8-Diaminooctane 1,9-Diaminononane 1,10-Diaminodecane $H_2N(CH_2)_nNH_2$
	Ferulic acid 
	3,4-Dimethoxycinnamic acid 
	Sinapinic acid 
Feruloylputrescine 	Feruloylcadaverine 
	Diferuloylputrescine 
	Diferuloylcadaverine 

(C4), 1,5-diaminopentane (C5), 1,6-diaminohexane (C6), 1,7-diaminoheptane (C7), 1,8-diaminooctane (C8), 1,9-diaminononane (C9) and 1,10-diaminodecane (C10) (Tokyo-kasei). Ferulic acid (Kishida) was also used. Feruloylputrescine (FP), feruloylcadaverine, diferuloylputrescine, diferuloylcadaverine, 3,4-dimethoxycinnamic acid and sinapinic acid were synthesized at the Ome Research Laboratories of Tobishi Pharmaceutical Co., Ltd. (Tokyo).

Of these compounds, C4, C5 and FP were isolated from the water-soluble extract of pollen (T-60) and identified (Table 1). T-60 was extracted from the mixture of the pollen of *Zea mays, Phleum pratense, Secale cerale, Pinus montana,* etc.; and 26% of the mixture was the pollen of corn (*Zea mays*) (9).

FP, ferulic acid and related compounds were dissolved in 0.5% sodium carbonate solution, and the other chemicals were dissolved in distilled water. All were diluted with distilled water.

EC50 and IC50 values with 95% confidence limits were calculated using the probit method.

Results

Effects of diamines on bladder smooth muscle: C7, C8, C9 and C10 (3–10 mM) markedly contracted bladder strips in a concentration-dependent manner (Fig. 1). These four diamines (30 mM) induced substantial contractions, equivalent to 20.5±2.6% (n=6), 29.2±1.1% (n=6), 64.6±8.6% (n=6) and 93.2±9.3% (n=6) of the ACh (55 μM)-induced response, respectively. The ACh (55 μM)-induced contraction corresponded to 36% of the maximal response (9).

On the other hand, C2, C3, C4, C5 and C6

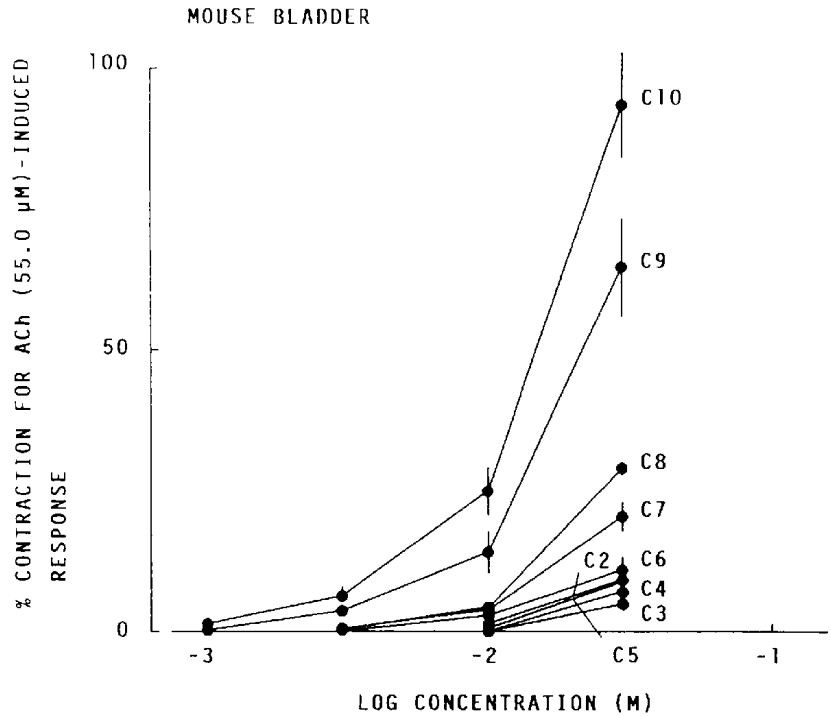


Fig. 1. Log cumulative concentration-response curve for contraction of the mouse bladder strips induced by diamines (C2–C10). C2, C3, C4, C5, C6, C7, C8, C9 and C10 represent 1,2-diaminoethane, 1,3-diaminopropane, 1,4-diaminobutane, 1,5-diaminopentane, 1,6-diaminohexane, 1,7-diaminoheptane, 1,8-diaminooctane, 1,9-diaminononane and 1,10-diaminodecane, respectively. Contractions are expressed as percentages of the contraction induced by acetylcholine (ACh, 55 μM). Each point represents the mean (n=6) with vertical lines indicating S.E.M.

only slightly contracted the bladder strips at 10–30 mM. These five diamines (30 mM) induced contraction equivalent to 8.6±0.8% (n=6), 5.0±1.1% (n=6), 7.6±1.3% (n=6), 8.4±0.8% (n=6) and 11.2±2.1% (n=6) of the ACh (55 μM)-induced response, respectively. These results indicate that C4 and C5, pollen-extract components, had little effect on bladder smooth muscle.

Effects of diamines on urethral smooth muscle: C7, C8, C9 and C10 potentiated the noradrenaline (NA, 1.77 μM)-induced contraction of urethral strips in a concentration-dependent manner (Fig. 2). The concentration of NA was near to the EC50 in the NA concentration-response curve. These effects became maximal at 1.0–3.0 mM, with C7, C8, C9 and C10 potentiating the contractions by 22.2±2.1% (n=6), 46.0±7.9% (n=6), 73.8±17.0% (n=6) and 71.5±7.3% (n=6), respectively. The respective IC50 values (with 95% confidence limits) were 115 (83–160) μM, 151 (61–377) μM, 124 (81–191) μM and 69 (52–92) μM. These diamines not only potentiated NA-induced contraction, but slightly contracted the urethral strips themselves (Fig. 2, dotted line). The maximal contraction induced by C7, C8, C9 and C10 at 0.3–1.0 mM was 0.7±0.5% (n=6), 1.3±0.7% (n=6), 10.9±4.5% (n=6) and 10.6±3.8% (n=6), respectively, of the NA-induced contraction.

In contrast, C2, C3, C4, C5 and C6 (0.3–30 mM) inhibited the NA-induced contraction of urethral stips in a concentration-dependent manner (Fig. 3). C2, C5 and C6 at concentrations of 30 mM completely inhibited it by 74.1±2.6% (n=6) and 58.0±3.0% (n=6) at the same concentrations. IC50 values (with 95% confidence limits) were 4.8

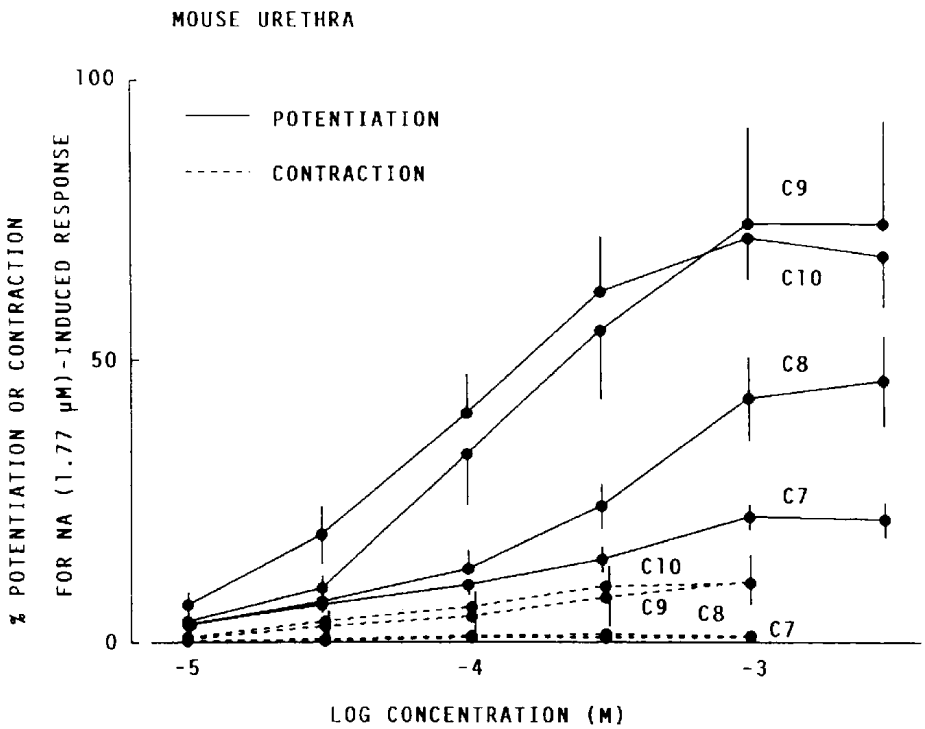


Fig. 2. Log cumulative concentration-response curve for potentiation of noradrenaline (NA, 1.77 μM)-induced contraction and for contraction induced by diamines (C7–C10) in mouse urethral strips. C7, C8, C9 and C10 represent 1,7-diaminoheptane, 1,8-diaminooctane, 1,9-diaminononane and 1,10-diaminodecane, respectively. Potentiations (solid lines) and contractions (dotted lines) are expressed as percentages of the contraction induced by NA. Each point represents the mean (n=6) with vertical lines indicating S.E.M.

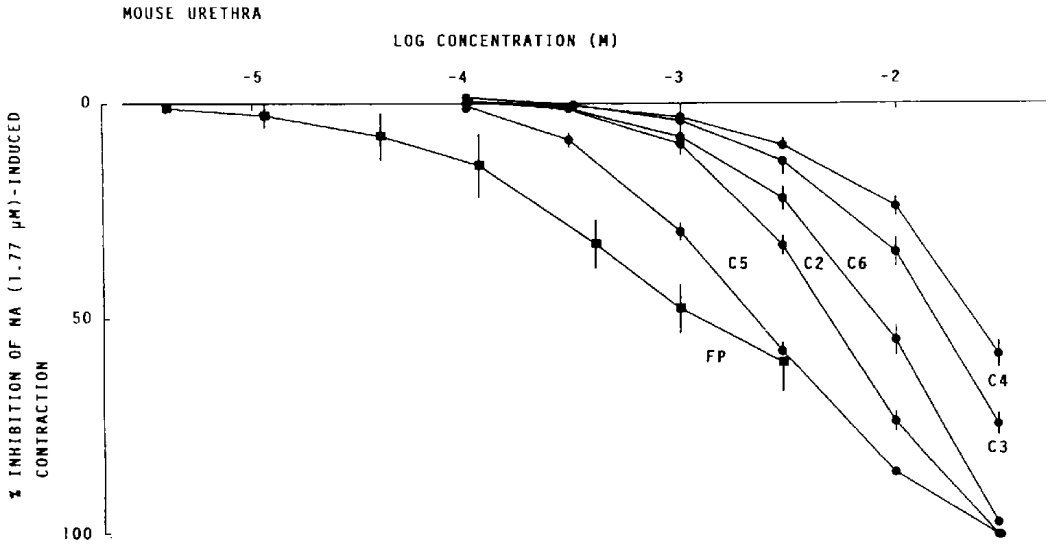


Fig. 3. Log cumulative concentration-response curve for inhibition by diamines (C2–C6) and feruloylputrescine (FP) of noradrenaline (NA, 1.77 μM)-induced contraction of mouse urethral strips. C2, C3, C4, C5 and C6 indicate the same compounds as in Fig. 1. Inhibitions are expressed as percentages of the contraction induced by NA. Each point represents the mean (n=6) with vertical lines indicating S.E.M.

Table 2. Inhibition of noradrenaline (1.77 μM)-induced contraction by diamines and their derivatives in mouse urethral smooth muscle

Compound	Concentration (mM)	% Inhibition (n)[a]
Putrescine (C4)[b]	1.0	3.1±1.4 (6)
Cadaverine (C5)[b]	1.0	29.6±1.9 (6)
Ferulic acid	0.515	3.8±1.3 (5)
3,4-Dimethoxycinnamic acid	0.480	26.8±5.8* (5)
Sinapinic acid	0.446	26.1±6.3** (5)
Feruloylputrescine[b]	0.378	32.5±5.5** (5)
Feruloylcadaverine	0.359	46.3±7.1** (5)
Diferuloylputrescine	0.227	13.5±4.8* (5)
Diferuloylcadaverine	0.220	14.2±2.8** (5)

Contractions induced by noradrenaline (1.77 μM) in the absence of the compounds were used as the control (=100%). Each value represents the mean±S.E.M. *$P<0.05$, **$P<0.01$: Significantly different from the control on the basis of the paired Student's t-test. [a]Number of observations. [b]Constituents of pollen-extract.

(3.9–6.2) mM, 14.2 (6.2–32.6) mM, 25.8 (11.9–56.0) mM, 2.3 (2.0–2.6) mM and 5.4 (2.7–10.9) mM, respectively, for C2, C3, C4, C5 and C6. These results indicate that the inhibitory effect of C5, a component of pollen-extract, is most potent among the diamines investigated.

Effects of FP and related compounds on bladder and urethral smooth muscle: FP, a component of pollen-extract, at 3.78–378 μM, concentration-dependently inhibited the NA-induced contraction of urethral smooth muscle (Fig. 3). The inhibitory effect of FP at 378 μM was 32.5±5.5% (n=5), while C5 inhibited the contraction by 29.6±1.9% (n=6) at 1.0 mM (Table 2). FP (0.3–3.0 mM) suppressed the maximal response of NA-induced contraction without shifting the concentra-

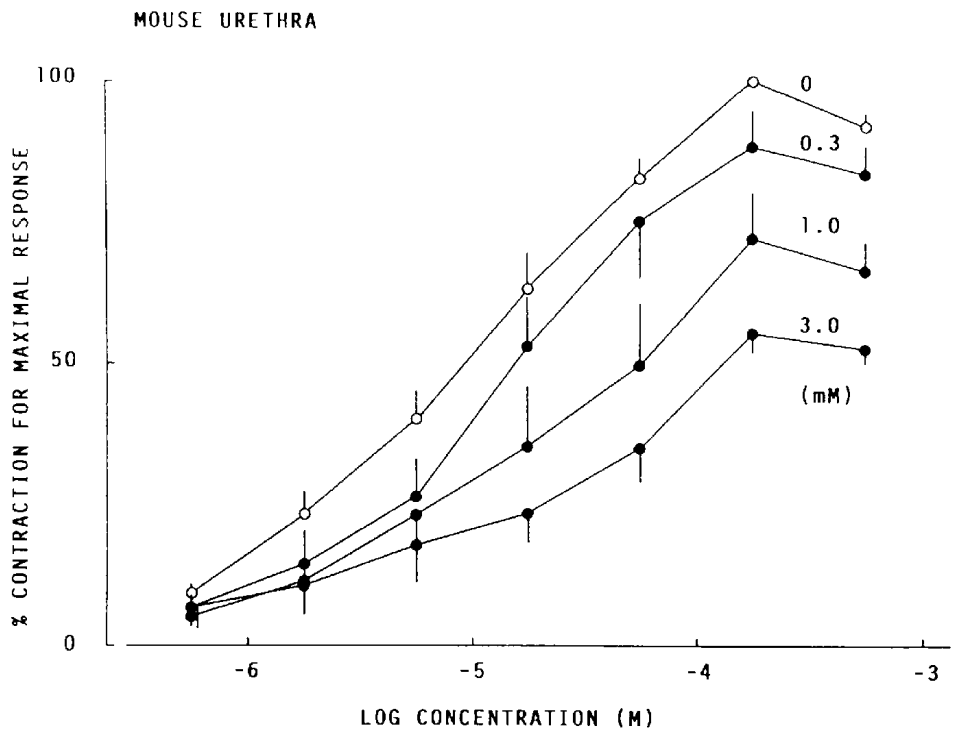


Fig. 4. Log cumulative concentration-response curve for contraction of mouse urethral strips induced by noradrenaline (NA) in the presence of feruloylputrescine (0, 0.3, 1.0 and 3.0 mM). Contractions are expressed as percentages of the maximal contraction induced by NA (177 μM) alone. Each point represents the mean (n=4–12) with vertical lines indicating S.E.M.

tion-response curve (Fig. 4). FP (3.78–378 μM) had little effect on bladder contraction.

Compounds related to FP (feruloylcadaverine, diferuloylputrescine, diferuloylcadaverine, ferulic acid, 3,4-dimethoxycinnamic acid, sinapinic acid) inhibited the NA-induced contraction of urethral smooth muscle (Table 2), while these compounds at 0.22–0.45 mM had little effect on bladder smooth muscle (data not shown). The inhibitory effect of feruloylcadaverine (359 μM) was greater than that of FP (378 μM), while the effects of the other compounds were less than those of FP. These results indicate that feruloylcadaverine inhibited the NA-induced contraction of urethral smooth muscle to a greater extent than the other components.

Discussion

Polyamines are known to have various pharmacological effects on many tissues including an inhibitory effect on smooth muscle. These effects are partially explained on the basis of the cationic nature of polyamines (4, 11, 12), a similarity of their effects to Ca^{2+}-entry blockers (8) and their inhibitory effects on protein kinase C (13).

In this study, we found that components of pollen-extract, C4 and C5, and diamines with shorter carbon chains (C2–C5) inhibited the NA-induced contraction of urethral smooth muscle. Among the nine diamines investigated, C5 inhibited most effectively the NA-induced contraction of the urethra, suggesting that C5 is one of the active components in pollen-extract.

The diamines with longer carbon chains (C7–C10) had potentiating effects and slight contractile effects on urethral smooth muscle, and they also had contractile effects on bladder smooth muscle. The excitatory mechanism of polyamines is as yet not well defined. Recently, polyamines have been speculated to have a regulatory effect on calcium fluxes (14,

15).

Concerning their potentiating effect on the urethra and contractile effect on the bladder, the order of potency was C10≧C9>C8>C7. A parallel relationship between the carbon chain length and the activity has been demonstrated with regard to these two effects. On the other hand, there was no relationship between the carbon chain lengths of C2, C3, C4, C5 and C6 and their inhibitory effect on NA-induced contraction of the urethra.

FP can also be isolated from some flowering plants (16), and its hypotensive activity has already been reported in the cat, rabbit and dog (17, 18). The mechanism of this hypotensive effects has not been reported. In this study, we found that not only FP but also feruloylcadaverine had a relatively strong inhibitory effect on urethral contraction. Since it was the most effective of the compounds isolated and identified, FP is one of the active components in pollen-extract.

These results suggest that C5 and FP are active components in pollen-extract with respect to inhibition of NA-induced contraction of mouse urethra. We have previously reported that pollen-extract, which contains C4, C5 and FP (10), inhibits NA-induced contraction, partially, in a competitive manner (9). However, the inhibitory effect of FP was non-competitive with respect to NA. On the other hand, other components, like caffeoylferuloylspermidine and diferuloylspermidine, have been identified in the pollen of *Corylus avellana* (19). It is suggested that not only C5 and FP but also the other components may act to inhibit NA-induced contraction of the urethra.

In conclusion, the diamines with shorter carbon chains (C2–C6) and compounds related to FP had an inhibitory effect on the urethra and a weak contracting effect on the bladder. FP and C5 were most effective in the components of pollen extract with respect to inhibition of urethral contraction, resulting in facilitation of the discharge of urine in vivo.

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