Regular Articles

Alpha-adrenolytic Activities of N-Benzyl-β-phenethylamine Derivatives on Isolated Smooth Muscles

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Previously N-methyl-5,6,7,8-tetrahydrodibenz[c,e]azocine (DA-VIII-Me) and N-methyl-10,11-methylenedioxy-5,6,7,8-tetrahydrodibenz[c,e]azocine (DA-VIII-M-Me) were found to have strong α-adrenolytic activities;[2] the pA_2 values were 8.76 and 7.71, respectively. N-Benzyl-N-methyl-β-phenethylamine (BMPA) and N-benzyl-N-methyl-3,4-methylenedioxy-β-phenethylamine (BMPA-MO) which resemble DA-VIII-Me and DA-VIII-M-Me in structure but with the azocine ring opened at the biphenyl bond, were synthesized. It was found that the α-adrenolytic activities of these compounds on rat aortic strips were much less than those of DA-VIII-Me and DA-VIII-M-Me; the pA_2 values for BMPA and BMPA-MO were 6.79 and 5.78, respectively. Thus it was concluded that the dibenz[c,e]azocine structure of the latter compounds was important for their α-adrenolytic activities.

It was also found that BMPA and BMPA-MO had a dual action, producing a moderately strong long-lasting contraction and inhibiting the contractile responses to various stimulants of isolated smooth muscles.

Keywords——α-adrenolytic activity; anti-5-HT activity; dibenz[c,e]azocine; N-benzyl-β-phenethylamine derivatives; rat aortic strip; guinea-pig ileum

Previously we[2] reported that compounds having a tetrahydrodibenz[c,e]azocine structure show various extents of α-adrenolytic activity on rat aortic strips; among the 15 compounds tested, N-methyl-5,6,7,8-tetrahydrodibenz[c,e]azocine (DA–VIII–Me) had more potent α-adrenolytic activity than phentolamine, and N-methyl-10,11-methylenedioxy-5,6,7,8-tetrahydrodibenz[c,e]azocine (DA–VIII–M–Me) had almost the same activity as phentolamine.

To investigate whether the azocine structure of an 8-member ring in these compounds is important for their α-adrenolytic activity, we synthesized N-benzyl-N-methyl-β-phenethylamine (BMPA) and N-benzyl-N-methyl-3,4-methylenedioxy-β-phenethylamine (BMPA–MO), which have structures similar to those of DA–VIII–Me and DA–VIII–M–Me but with the azocine ring opened at the biphenyl bond, as Chart 1 shows, and tested their α-adrenolytic activities on rat aortic strips.

It was also found that BMPA and BMPA–MO at high concentrations produced moderately strong long-lasting contractions of isolated longitudinal smooth muscle of guinea pig ileum or rat aortic strips.

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1) Location: *Shomachi 1-Chome, Tokushima, 770, Japan.*

2) Y. Ishida, K. Watanabe, S. Kobayashi, and M. Kihara, *Chem. Pharm. Bull.* (Tokyo), **25**, 1851 (1977).
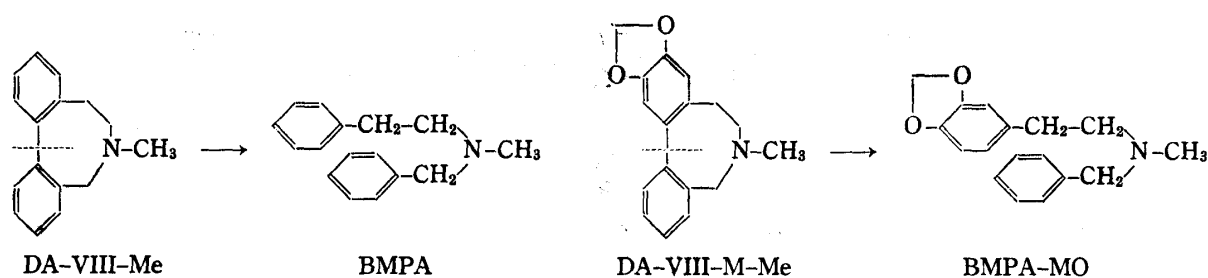
Chart 1

DA-VIII-Me   BMPA   DA-VIII-M-Me   BMPA-MO

Methods and Materials

Pharmacological Actions——a) Strips of thoracic aorta were cut spirally from rats weighing 200 to 250 g, as described by Furchgott and Bhadrakom[3] and suspended in a 10 ml organ bath in modified Krebs-bicarbonate solution (composition in mM; NaCl, 118.2; KCl, 4.6; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 24.8 and glucose, 10) at 36° bubbled with 5% CO_2 in oxygen. Contractions were recorded on the smoked drum of a kumograph with an isotonic lever weighing about 1.0 g. A cumulative method[4] was used and inhibitory activity was estimated by recording the response curves for each agonist in the absence and presence of an antagonist. After equilibrating the strips for 2 hr with repeated washing, the cumulative response curve to an agonist was recorded 2 to 3 times at intervals of 30 min until the responses were the same in two successive recordings either in the absence or presence of antagonist. In each recording the exposured time of the strip to the antagonist was 15 min. Contractions were expressed as percentages of the maximal response to each agonist; the maximal responses to different agonists were not significantly different. The parameters of drugs were calculated by the method of Van Rossum;[4] the pA_2 value is defined as the negative logarithm of the molar concentration of a competitive antagonist shifting the dose-response curve of an agonist to the right by 0.3 unit, and the pD_2' value as the negative logarithm of the molar concentration of a noncompetitive antagonist reducing the maximal effect of an agonist by 50%.[5]

b) Longitudinal muscle strips from guinea pig ileum were prepared as described by Rang[6] and suspended in a 10 ml organ bath in Tyrode solution (composition in mM: NaCl, 137; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 1.1; NaH_2PO_4, 0.4; $NaHCO_3$, 11.9 and glucose, 10) at 32° bubbled with air. Contractions and tensions were recorded isotonically or isometrically using an isotonic lever with a 0.3 g weight or a force displacement transducer recording in ink on a polygraph (Nihon Kohden Co.).

Syntheses of BMPA and BMPA-MO——a) N-Benzyl-N-methyl-β-phenethylamine (BMPA): Benzaldehyde (1.6 g) was heated with β-phenethylamine (0.9 g) at 105° for 30 min to give the Schiff's base (1.86 g). Reduction of the base (1.86 g) in $EtOH-CHCl_3$ (3: 2) (180 ml) with $NaBH_4$ (4.0 g) at room temperature gave N-benzyl-β-phenethylamine (434 mg) as an oil, which was identified by its nuclear magnetic resonance (NMR) spectrum [$CDCl_3$, δ: 7.44—7.03 (10H, m, aromatic H), 3.76 (2H, s, $ArCH_2N$), 2.84 (4H, m, $ArCH_2CH_2N$), 1.45 (1H, s, NH)] and elementary analysis of its hydrochloride, mp 254—257° (dec.) (*Anal.* Calcd. for $C_{15}H_{17}N \cdot HCl$: C, 72.71; H, 7.32; N, 5.65. Found: C, 72.91; H, 7.43; N, 5.62). Methylation of the β-phenethylamine (410 mg) with formalin (2.07 ml), boric acid (23 mg), and $NaBH_4$ (700 mg) and purification of the resulting crude product by preparative thin–layer chromatography (TLC) using SiO_2 [acetone-$CHCl_3$ (1: 10)] gave N-benzyl-N-methyl-β-phenethylamine (257 mg, 16.3% overall yield from β-phenethylamine) as a colorless oil.[7] *Anal.* Calcd. for $C_{16}H_{19}N$: C, 85.28; H, 8.50; N, 6.22. Found: C, 84.94; H, 8.43; N, 6.34. NMR ($CDCl_3$) δ: 7.40—7.00 (10H, m, aromatic H), 3.56 (2H, s, $ArCH_2N$), 2.96—2.52 (4H, m, $ArCH_2CH_2N$), 2.28 (3H, s, NCH_3).

b) N-Benzyl-N-methyl-3,4-methylenedioxy-β-phenethylamine (BMPA-MO): (i) 3,4-Methylenedioxy-β-nitrostyrene: Piperonal (10 g) and *n*-butylamine (61 ml) were heated at 95° for 1 hr. The resulting residue was mixed with AcOH (50 ml) and CH_3NO_2 (13 ml) and allowed to stand overnight at room temperature. Working up in the usual way gave yellow needles (7.2 g, 55.9%) of 3,4-methylenedioxy-β-nitrostyrene. *Anal.* Calcd. for $C_9H_7NO_4$: C, 55.96; H, 3.65; N, 7.25. Found: C, 55.92; H, 3.61; N, 7.30. IR ν_{max}^{KBr} cm^{-1}: 1630 (C=C). NMR ($CDCl_3$) δ: 7.90 (1H, d, $J=14$ Hz, ArCH=$CHNO_2$), 7.40 (1H, d, $J=14$ Hz, ArCH=$CHNO_2$), 7.05 (1H, dd, $J=8$ and 1 Hz, C-6-H), 6.98 (1H, d, $J=1$ Hz, C-2-H), 6.82 (1H, d, $J=8$ Hz, C-5-H), 6.01 (2H, s, OCH_2O).

3) R.F. Furchgott and S. Bhadrakom, *J. Pharmacol. Exp. Ther.*, **108**, 129 (1953).
4) J.M. Van Rossum, *Arch. Int. Pharmacodyn.*, **143**, 299 (1963).
5) E.J. Ariens and J.M. Van Rossum, *Arch. Int. Pharmacodyn.*, **109**, 468; **110**, 275 (1957).
6) H.P. Rang, *Br. J. Pharmac.*, **22**, 356 (1964).
7) A.R. Lepley and A.G. Giumaini, *J. Org. Chem.*, **32**, 1706 (1969).

(ii) N-Benzyl-3,4-methylenedioxy-β-phenethylamine: A solution of the nitrostyrene (602 mg) in EtOH (140 ml)–MeOH (30 ml) and zinc amalgam [prepared from 5% $HgCl_2$ (41 ml) and zinc dust (1.5 g)] were added to a mixture of conc. HCl (3.5 ml) and MeOH (10 ml) at 50°, and the reaction mixture was stirred for 1.5 hr. Working up in the usual manner gave 3,4-methylenedioxy-β-phenethylamine (236 mg, 46.7%) as an oil. NMR ($CDCl_3$) δ: 6.80—6.47 (3H, m, aromatic H), 5.87 (2H, s, OCH_2O), 3.03—2.38 (4H, m, $ArCH_2CH_2N$), 1.83 (2H, s, NH_2).

The β-phenethylamine (236 mg) and benzaldehyde (4.5 ml) were heated in a sealed tube at 110° for 3 hr. Concentration of the mixture gave an oil (409 mg), which was dissolved in EtOH–$CHCl_3$ (3: 2, by volume) (40 ml). Reduction of the solution with $NaBH_4$ (355 mg) gave N-benzyl-3,4-methylenedioxy-β-phenethyl-amine (341 mg) as an oil. NMR ($CDCl_3$) δ: 7.40—6.80 (8H, m, aromatic H), 5.80 (2H, s, OCH_2O), 3.79 (2H, s, $ArCH_2N$), 3.00—2.60 (4H, m, $ArCH_2CH_2N$), 1.52 (1H, s, NH). The oil was crystallized as its hydrochloride (173 mg, 47.7% from 3,4-methylenedioxy-β-phenethylamine), mp 216—217° (from acetone). $Anal.$ Calcd. for $C_{16}H_{17}NO_2 \cdot HCl$: C, 65.86; H, 6.25; N, 4.80. Found: C, 65.84; H, 6.17; N, 5.02.

(iii) N-Benzyl-N-methyl-3,4-methylenedioxy-β-phenethylamine (BMPA–MO): To a mixture of β-phenethylamine (123 mg), boric acid (6 mg), formalin (578 mg) in MeOH (4 ml) was added $NaBH_4$ (182 mg). The mixture was stirred for 1 hr and then AcOH (0.5 ml) and H_2O (0.3 ml) were added. Working up in the usual way gave the N-methylated product (116 mg, 89.4%) as an oil. NMR ($CDCl_3$) δ: 7.10—6.75 (8H, m, aromatic H), 5.87 (2H, s, OCH_2O), 3.56 (2H, s, $ArCH_2N$), 2.90—2.50 (4H, m, $ArCH_2CH_2N$), 2.27 (3H, s, NCH_3). The oil was converted to its hydrochloride (white needles), mp 164—167° (from acetone–MeOH). $Anal.$ Calcd. for $C_{17}H_{19}NO_2 \cdot HCl \cdot 1/4H_2O$: C, 65.84; H, 6.66; N, 4.46. Found: C, 66.19; H, 6.49; N, 4.50.

Results

1. α-Adrenolytic Activities of BMPA and BMPA-MO

Figure 1 shows the dose-response curves of isolated rat aortic strips to adrenaline (a) and 5-HT (b) in the absence and presence of BMPA. Preincubation of the strips with 3×10^{-6}, 10^{-5}, and 3×10^{-5} M BPMA shifted the dose-response curves for adrenaline to the right, without suppressing the maximal response significantly; preincubation of the strips with 10^{-5} and 3×10^{-5} M BMPA shifted the dose-response curves for 5-HT less and suppressed the maximal response. The pA_2 and pD'_2 values of BMPA against adrenaline and 5-HT are summarized in Table I. Preincubation with BMPA–MO caused contractions of the aortic strips to the extents indicated by the horizontal dotted lines in Fig. 2 (20—50% of the maximum with 10^{-5} M BMPA–MO). The extent of contraction was greatest with 10^{-5} M BMPA–MO and slightly less with 3×10^{-5} M BMPA–MO. This contraction was reversible, the original tonus being restored by washing the BMPA–MO out of organ bath. Because of this contraction with BMPA–MO alone, the pA_2 and pD'_2 values of BMPA–MO could not be estimated from the observed dose-response curves. Therefore, the dose-response curves for each agonist were calculated from the observed results, taking the extent of contraction in the presence of
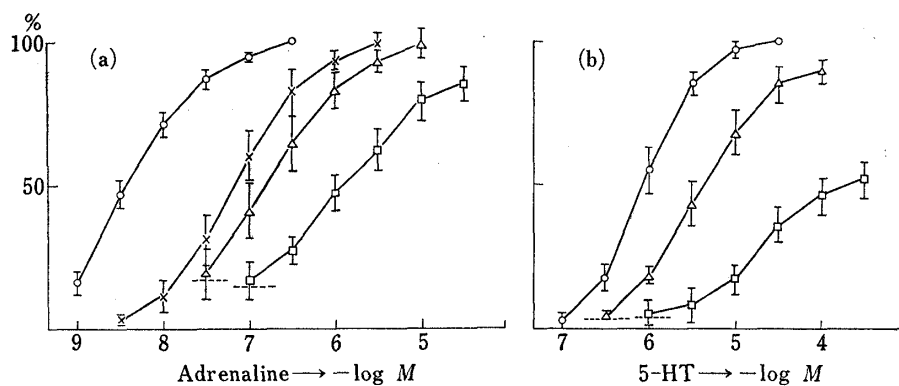

Fig. 1. Dose-response Curves of Rat Aortic Strips to Adrenaline (a) and 5-HT (b) in the Absence and Presence of BMPA

○——○: without BMPA, ×——×: with 3×10^{-6} M BMPA,
△——△: with 10^{-5} M BMPA, □——□: with 3×10^{-5} M BMPA.
The horizontal dotted lines indicate the contractions induced by BMPA alone.
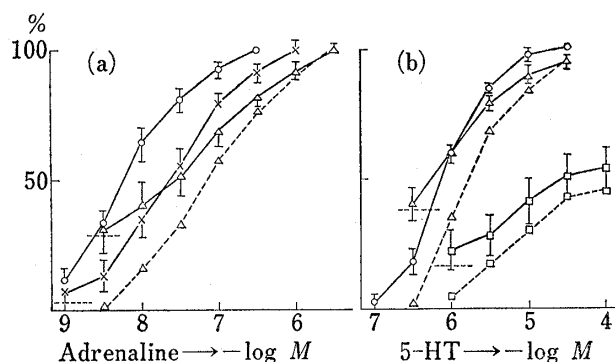
Fig. 2. Dose-response Curves of Rat Aortic Strips to Adrenaline (a) and 5-HT (b) in the Absence and Presence of BMPA-MO

○——○: without BMPA-MO,
×——×: with 3×10^{-6} M BMPA-MO,
△——△: with 10^{-5} M BMPA-MO,
□——□: with 3×10^{-5} M BMPA-MO.

The horizontal dotted lines indicate the contractions induced by BMPA-MO alone and the dotted curves were calculated as described in the text.

BMPA–MO as 0% (Fig. 2). The pA_2 and pD'_2 values of BMPA–MO against adrenaline and 5-HT obtained from these curves are summarized in Table I. For 5-HT, the pD_2' values were estimated from the curves with 3×10^{-5} M BMPA and BMPA–MO, but the pA_2 values had to be estimated from the curves with 10^{-5} M antagonists because those with 3×10^{-5} M antagonists were too suppressed to make it possible to estimate the degree of parallel shift to the right. BMPA also caused some contraction (up to 10% of the maximum), although so much less than BMPA–MO, in determining the parameters, correction of the curves in the presence of BMPA was not necessary.

As the contractions induced by BMPA and BMPA–MO were not influenced by pretreatment with 3×10^{-7} M atropine, 3×10^{-7} M phentolamine or 10^{-7} g/ml tetrodotoxin, they did not seem to be due to liberation of some neurotransmitters.

TABLE I. α-Adrenolytic and Anti-5-HT Activities of BMPA and BMPA-MO on Rat Aortic Strips

| Antagonists | | Agonists | | Ratio of activities[b] |
		Adrenaline	5-HT	
BMPA	pA_2	$6.79 \pm 0.08(15)$[a]	$5.60 \pm 0.19(4)$	15.5
	pD_2'	No effect[c]	$4.46 \pm 0.08(4)$	
BMPA-MO	pA_2	$5.78 \pm 0.05(12)$	$5.12 \pm 0.17(4)$	4.6
	pD_2'	No effect	$4.57 \pm 0.09(4)$	

a) Mean±S.E., (Number of experiments).
b) Anti-logarithm of the difference between the pA_2 values against adrenaline and 5-HT.
c) At concentrations of up to 10^{-5} M.

2. Effects of BMPA and BMPA-MO on Isolated Longitudinal Muscle of Guinea Pig Ileum

Concentrations of over 10^{-5} M BMPA and BMPA-MO produced moderate, long-lasting contractions of isolated longitudinal muscle of guinea pig ileum, the effect of BMPA-MO being much stronger than that of BMPA. These effects were not influenced by 10^{-7} M atropine, 3×10^{-6} M hexamethonium or 3×10^{-7} g/ml tetrodotoxin. After longer preincubation (15—30 min) with BMPA-MO, the isometric tension and isotonic contraction of the ileum in response to ACh were both greatly reduced, as Fig. 3 shows.
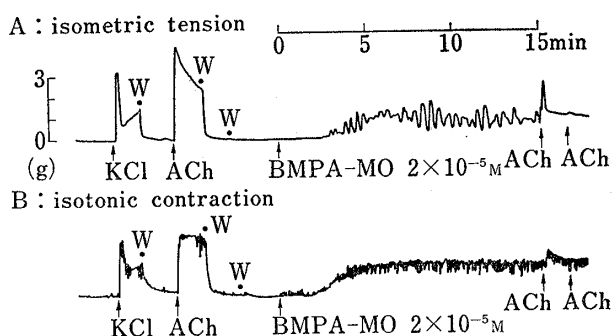

Fig. 3. An Example of the Dual Action of 2×10^{-5} M BMPA-MO on Isolated Longitudinal Muscle of Guinea Pig Ileum

Upper panel (A): isometric tension; lower panel (B): isotonic contraction. ACh: 10^{-5} M, KCl: 40 mM.

Note that the stimulating action of BMPA–MO appears in a few minutes after its application and the response to ACh 10^{-5} M was greatly inhibited at 15 min after the stimulation.

BMPA also had the dual action, but its stimulating activity was smaller than that of BMPA–MO. An example of the dual action of BMPA was shown in Fig. 4, in which the cumulative dose-response to ACh was inhibited by treatment with 10^{-5} M BMPA for 15 min. On the repeated application of BMPA the stimulating and inhibitory activities became stronger as Fig. 4 shows.
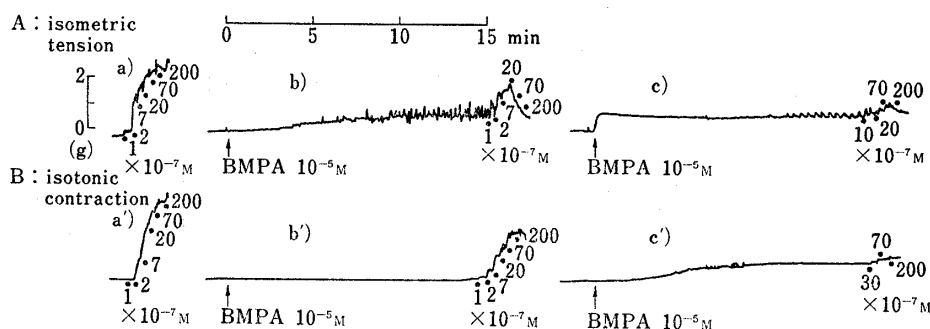

Fig. 4. An Example of the Dual Action of 10^{-5} M BMPA on Isolated Longitudinal Muscle of Guinea Pig Ileum

Upper panel (A): isometric tension; lower panel (B): isotonic contraction.
Note that the cumulative dose-response to ACh (a, a') is inhibited by BMPA 10^{-5} M after its stimulating action (b, b') and the stimulating and inhibitory activities become stronger on repeated application (c, c').

Discussion

In the present work we found that the α-adrenolytic activities of BMPA (pA_2=6.79± 0.08) and BMPA–MO (pA_2=5.78±0.05), which have similar structures to DA–VIII–Me (pA_2= 8.76±0.07)[2] and DA–VIII–M–Me (pA_2=7.71±0.05),[2] respectively, but without the azocine ring, show much weaker α-adrenolytic activities than the latter.

BMPA and BMPA–MO also inhibited the contractile responses to 5-HT and their effects seemed to be both competitive and non-competitive, because on progressive increase in concentration to 3×10^{-5} M the compounds shifted the dose-response curves for 5-HT to the right with decrease in the maximal contractions (Fig. 1b and Fig. 2b). The pA_2 values in the actions of BMPA and BMPA–MO against 5-HT were 5.63±0.19 and 5.12±0.17 and the pD_2' values were 4.46±0.08 and 4.57±0.09, respectively on rat aortic strips. Our previous report[2] showed that the pA_2 values of DA–VIII–Me and DA–VIII–M–Me against 5-HT were 6.00± 0.05 and 5.93±0.05, respectively. Thus the differences between the effects of DA–VIII–Me and BMPA, and DA–VIII–M–Me and BMPA–MO, respectively, against 5-HT were much smaller than the differences in their α-adrenolytic activities.

Krishnamurty[8] reported that phentolamine shifted the dose-response curves for noradrenaline and 5-HT to the right without suppressing the maximum rseponses and that the pA_2 values of phentolamine were 7.94±0.05 and 7.05±0.10 for norepinephrine and 5-HT, respectively. The differences between the pA_2 values of DA–VIII–Me (ΔpA_2=1.97) and DA–VIII–M–Me (ΔpA_2=1.69), respectively, against adrenaline and 5-HT are greater than the difference between the pA_2 values of phentolamine (ΔpA_2=0.89) against norepinephrine and 5-HT. Thus DA–VIII–Me and DA–VIII–M–Me have more specific α-adrenolytic actions than phentolamine.

Previously, in studies on irreversible blocking of the actions of adrenaline and 5-HT on rat aortic strips we[9] found that 6-(2-bromoethyl)-10,11-methylenedioxy-5,6,7,8-tetrahydro-

8) V.S.R. Krishnamurty, *Arch. Int. Pharmacodyn.*, **189**, 90 (1971).
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dibenz[c,e]azocine (DA–VIII–MBr), in which the nitrogen bears a 2-halogenoethylamine group, has a more selective α-adrenergic blocking action than dibenamine or phenoxybenzamine. Thus we concluded that the azocine structure in dibenz[c,e]azocine derivatives must be a component of the structural entity fitting the α-adrenoceptor either reversibly or irreversibly.

BMPA and BMPA–MO have other two effects: they produce a moderately strong, long-lasting contraction, and they inhibit the contractile responses of longitudinal muscle of guinea pig ileum or rat aortic strips to various stimulants. The moderate contractions are not due to liberation of some neurotransmitters, but probably to a direct action of these compounds on smooth muscle cells, judging from the present results. Recently, Ferrari et al.[10] showed that N-benzyl-salsolidine had a dual action in guinea pig taenia coli, its myolytic activity being associated with enhancement of the rate and amplitude of contractions. Later they found that papaverine,[11] qinidine, and ajamaline[12] had similar actions to that of N-benzyl-salsolidine. Our results show that the dual actions of BMPA and BMPA–MO are similar to that of N-benzyl-salsolidine. Further experiments are required on the details of their action mechanisms.

10) M. Ferrari, M. Furlanut, J. Maragno, E. Santi-Soncin, and Tóth, *Arch. Int. Pharmacodyn.*, 200, 40 (1972).
11) M. Ferrari and M. Furlanut, *Arch. Int. Pharmacodyn.*, 200, 57 (1972).
12) M. Ferrari, M. Furlanut, and I. Maragno, *Arch. Int. Pharmacodyn.*, 200, 64 (1972).