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Pharmacological profiles of new orally active nonpeptide tachykinin NK_1 receptor antagonists

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Abstract

Pharmacological profiles of new orally active amide-based tachykinin NK_1 receptor antagonists, *N*-[3,5-bis(trifluoromethyl)benzyl]-5-(4-fluorophenyl)-7,8-dihydro-*N*,7-dimethyl-8-oxo-1,7-naphthyridine-6-carboxamide (referred to as compound I) and two related compounds (compounds II and III), were compared with that of (+)-(2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine (CP-99,994), another nonpeptide tachykinin NK_1 receptor antagonist. Compounds I, II, III and CP-99,994 caused parallel rightward shifts of the concentration–response curve of substance P in the guinea-pig ileum pretreated with atropine, mepyramine and indomethacin, with the pA_2 values of 8.70, 7.56, 8.41 and 8.27, respectively. These antagonists did not alter the concentration–response curve of acetylcholine in the guinea-pig ileum nor that of neurokinin A in the rat vas deferens. Furthermore, contractile responses to senktide of the rat portal vein were not affected by these antagonists. In the isolated neonatal gerbil spinal cord pretreated with tetrodotoxin, substance P produced a dose-dependent depolarization of ventral roots. Compounds I, II, III and CP-99,994 caused parallel rightward shifts of the concentration–response curve of substance P in the spinal cord with the pA_2 values of 7.07, 5.93, 6.40 and 7.26, respectively. In contrast, these antagonists did not affect the concentration–response curve of L-glutamate. These results suggest that compounds I, II and III are selective antagonists for tachykinin NK_1 receptor both in peripheral tissues and the central nervous system. © 1998 Elsevier Science B.V.

Keywords: Ileum, guinea-pig; Portal vein, rat; Vas deferens, rat; Spinal cord; Substance P; Tachykinin receptor antagonist

1. Introduction

A number of selective nonpeptide tachykinin NK_1 receptor antagonists have recently been reported. These include CP-96,345 (Snider et al., 1991; Lembeck et al., 1992), RP 67580 (Garret et al., 1991), CP-99,994 (McLean et al., 1993a,b), SR 140333 (Emonds-Alt et al., 1993), CGP49823 (Vassout et al., 1994), RPR100893 (Lee et al., 1994; Tabart and Peyronel, 1994), L-732,138 (MacLeod et al., 1993), FK224 (Morimoto et al., 1992), FK888 (Fujii et al., 1992), LY303870 (Gitter et al., 1995) and CP-122,721 (McLean et al., 1996). Some of them are effective by oral administration (e.g. CP-99,994, RPR 100893, FK888 and CP-122,721).

Dietl and Palacios (1991) examined the phylogeny of tachykinin receptor localization in the vertebrate central nervous system and suggested that tachykinin NK_1 receptors are predominant in the human brain. Tachykinin NK_1 receptors are also widely distributed in peripheral tissues (Otsuka and Yoshioka, 1993). Therefore, it is expected that some tachykinin NK_1 receptor antagonists may be useful for clinical applications in the treatments of various ailments, such as pain, inflammation, arthritis, respiratory, gastrointestinal, urinary and psychotic disorders.

Recently, Natsugari et al. (1995a) synthesized a series of amide-based compounds in an attempt to develop tachykinin NK_1 receptor antagonists. As a result some potent orally active tachykinin NK_1 receptor antagonists were obtained, among which *N*-[3,5-bis(trifluoromethyl)-benzyl]-5-(4-fluorophenyl)-7,8-dihydro-*N*,7-dimethyl-8-oxo-1,7-naphthyridine-6-carboxamide (Fig. 1, referred to as 30a in Natsugari et al., 1995a and as compound I in this paper), is highly potent. In the present study, we describe the pharmacological profiles of Compound I and two

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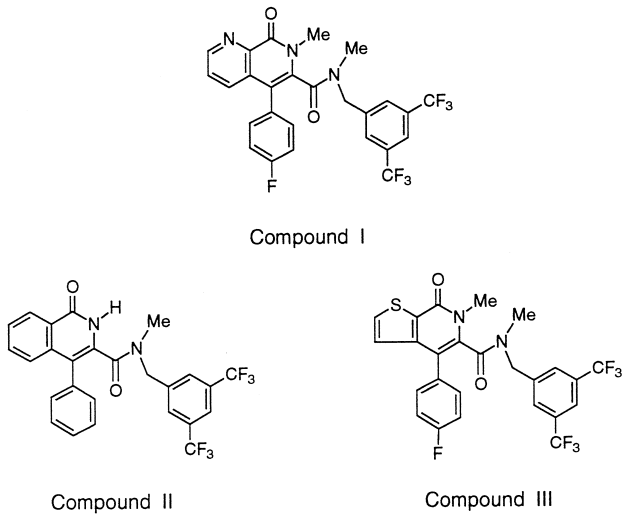

Fig. 1. Chemical structures of compounds I, II and III. After Natsugari et al. (1994, 1995a,b).

related compounds, *N*-[3,5-bis(trifluoromethyl)benzyl]-1,2-dihydro-*N*-methyl-1-oxo-4-phenyl-3-isoquinolinecarboxamide and *N*-[3,5-bis(trifluoromethyl)benzyl]-5-(4-fluorophenyl)-6, 7-dihydro-*N*,6-dimethyl-7-oxothieno[2,3-c]pyridine-5-carboxamide (Fig. 1, Natsugari et al., 1994, 1995b; referred to as compounds II and III, respectively, in this paper), as examined in the isolated guinea-pig ileum, the rat vas deferens and the rat portal vein, which are known to be predominantly endowed with tachykinin NK_1, NK_2 and NK_3 receptors, respectively (Regoli et al., 1988). In addition, to examine the actions of these compounds on tachykinin receptors in the central nervous system, isolated gerbil spinal cord preparation was used. Gerbils were used because compound I and related compounds were reported to be active on human tachykinin NK_1 receptors and gerbils belong to the same group of species as humans with respect to the actions of tachykinin NK_1 receptor antagonists (see Section 4). As a comparison, (+)-(2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine (CP-99,994), a recently reported nonpeptide tachykinin NK_1 receptor antagonist which is active on human tachykinin NK_1 receptors, was examined in parallel for its pharmacological properties.

2. Materials and methods

2.1. Guinea-pig ileum

Hartley guinea-pigs of either sex weighing 200 to 300 g were stunned by a blow on the head and killed by exsanguination. The ileum was excised rapidly and the terminal portion of the ileum was used, after discarding the 5–10 cm segment of the distal ileum. A segment of 1.5–2 cm in length was suspended in an organ bath containing 5 ml Tyrode solution at 37°C under a tension of about 1 g. Contractile responses were recorded isotonically. The medium in the bath was bubbled with a mixture of 95% O_2 and 5% CO_2. The composition of the Tyrode solution was (mM): NaCl, 137.9; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 0.5; $NaHCO_3$, 11.9; NaH_2PO_4, 0.5 and glucose, 5.6. In the experiments in which effects of tachykinin receptor antagonists on the action of substance P were examined, the ileum was treated with atropine 4 μM, mepyramine 4 μM and indomethacin 3.6 μM in order to block the actions of acetylcholine and histamine and the production of prostaglandins (Lee et al., 1982; Dion et al., 1987). Under such conditions, the contractile effect of substance P is mostly due to its action on tachykinin NK_1 receptors (Holzer and Lembeck, 1980; Laufer et al., 1985). To construct concentration–response curves, substance P was applied cumulatively first in the absence of the antagonists, then the preparation was pretreated with one of the antagonists for 10 min and substance P was again applied cumulatively in the presence of the antagonist. The pA_2 values and slopes of Schild plots were determined as described by Arunlakshana and Schild (1959). In order to examine the receptor specificity of the antagonists, their effects on the concentration–response curves of acetylcholine were examined in normal Tyrode solution.

2.2. Rat vas deferens

Male Wistar rats weighing 200–300 g were stunned by a blow on the head and killed by exsanguination. Segments of the vas deferens were dissected. Other experimental details were the same as described for the guinea-pig ileum. Experiments were started after the preparation had been equilibrated for 30 min in Tyrode solution. A concentration–response curve of neurokinin A was first constructed in the normal Tyrode solution. Then, the preparation was treated with one of the antagonists for 10 min and the concentration–response curve of neurokinin A was again constructed in the presence of the antagonist.

2.3. Rat portal vein

Male Wistar rats weighing 200–300 g were stunned by a blow on the head and killed by exsanguination. A longitudinal strip of the portal vein was dissected removing surrounding tissues, as described by Mastrangelo et al. (1987). The strip was suspended in an organ bath containing 5 ml modified Krebs–Henseleit solution at 37°C under a tension of about 0.5 g and the medium was bubbled with a mixture of 95% O_2 and 5% CO_2. The composition of the modified Krebs–Henseleit solution was (mM): NaCl, 118.0; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; KH_2PO_4, 1.19 and glucose, 11.0. After the preparation was equilibrated for 60 min, mechanical responses were

recorded isotonically. Since the contractile responses to senktide gradually declined upon repeated application, a response to senktide at 10 nM, which corresponded approximately to EC_{50}, was recorded only once for each preparation with or without the pretreatment with the antagonist. The amplitudes of contractile responses were expressed as percentages of the maximal response to noradrenaline (10 μM). To test the effect of the antagonists, the preparation was pretreated with one of the antagonists for 10 min before senktide was applied.

2.4. Gerbil spinal cord

The spinal cord below thoracic segments was isolated from 1–4 day-old gerbils of both sex under ether anesthesia. The spinal cord was hemisected, placed in a bath of 0.2 ml volume and perfused with artificial cerebrospinal fluid (CSF) containing tetrodotoxin (0.3 μM) at a rate of 4 ml/min. The composition of the artificial CSF was (mM): NaCl, 138.6; KCl, 3.35; $CaCl_2$, 1.26; $MgCl_2$, 1.16; $NaHCO_3$, 21.0; NaH_2PO_4, 0.58 and glucose, 10.0. The perfusion medium was saturated with a gas mixture of 95% O_2 and 5% CO_2 before perfusion and maintained at 27°C in the perfusion bath. Potentials were recorded extracellularly from a lumbar ventral root (L3–L5) with a tightly fitting suction electrode and displayed via preamplifier on an oscilloscope and a d.c. pen-recorder.

Effects of the antagonists on the dose–response curves of substance P and L-glutamate were examined. The agonists were bath-applied for 30 s at 10 min intervals. A concentration–response curve of substance P or L-glutamate was first constructed in artificial CSF containing tetrodotoxin, and then the preparation was pretreated with one of the antagonists for 25 min and the concentration–response curve was again recorded in the presence of the antagonist.

2.5. Drugs

Compounds I, II, III (see Introduction) were provided by Dr. H. Natsugari, Pharmaceutical Research Division, Takeda Chemical Industries, Japan. CP-99,994, (+)-(2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine, was donated by Dr. A. Nagahisa, Central Research Division, Pfizer, Japan. Senktide (succinyl-[Asp^6, Me-Phe^8] substance P-(6–11); Papir-Kricheli et al., 1987) was a gift from Professor Z. Selinger, Department of Biological Chemistry, the Hebrew University of Jerusalem, Israel. Substance P and neurokinin A were purchased from Peptide Institute, Osaka, Japan; atropine sulfate and sodium L-glutamate from Wako, Japan; indomethacin from Sigma, USA and tetrodotoxin from Sankyo Co., Japan. Other drugs were obtained from various commercial sources.

3. Results

3.1. Effects of compounds I, II, III and CP-99,994 on tachykinin receptors in the peripheral tissues

Three isolated preparations, guinea-pig ileum, rat vas deferens and rat portal vein, were used to examine the actions and specificities of compounds I, II, III and CP-99,994 on tachykinin NK_1, NK_2 and NK_3 receptors (Regoli et al., 1988).

In the guinea-pig ileum, pD_2 of substance P was 8.38 ± 0.18 (mean ± S.E.M.; $n = 4$, Fig. 2). Addition of CP-

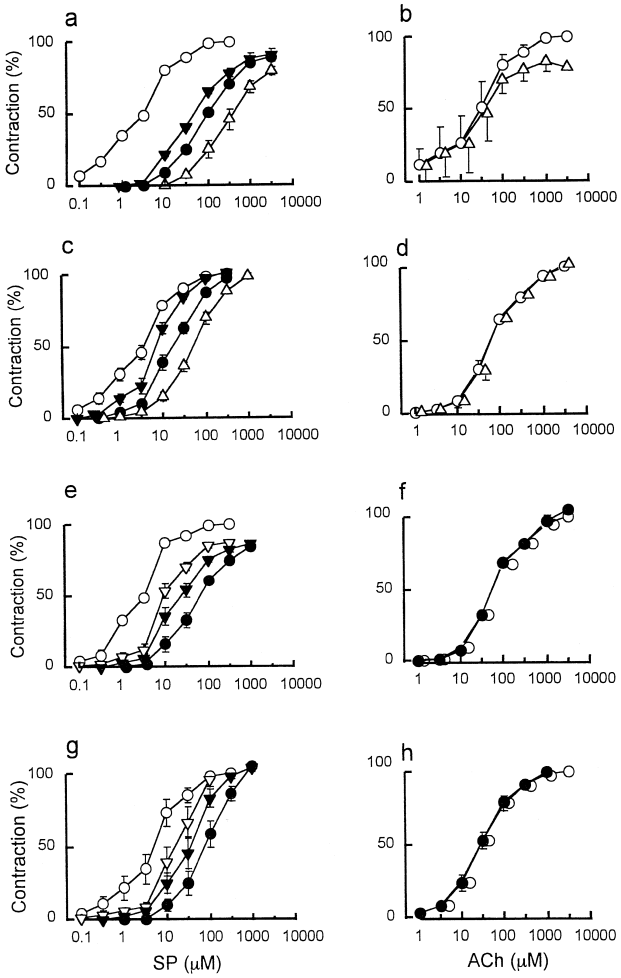

Fig. 2. Effects of compounds I, II, III and CP-99,994 on the concentration–response curves of substance P (SP) and acetylcholine (ACh) in the guinea-pig ileum. Ordinate: peak height of agonist-induced contractile response expressed as percentages of maximal control responses of each agonist. Abscissa: logarithmic molar concentration of each agonist. Each symbol and bar represent the mean ± S.E.M. of 3 to 4 observations. For some symbols, standard errors were smaller than the symbols and therefore not shown. Some symbols are displaced horizontally to avoid overlapping. (a, c, e and g) Concentration–response curves of substance P in the presence of atropine (4 μM), mepyramine (4 μM) and indomethacin (3.6 μM) and (b, d, f and h) concentration–response curves of acetylcholine in normal Tyrode solution. (a and b) Effects of compound I; (c and d) effects of compound II; (e and f) effects of compound III and (g and h) effects of CP-99,994. ○, control; ▽, ▼, ● and △, after addition of the tachykinin antagonists at 0.01, 0.03, 0.1 and 0.3 μM, respectively.

Table 1
pA_2 values and slopes of Schild plots for compounds I, II, III and CP-99,994 against substance P in the guinea-pig ileum and the neonatal gerbil spinal cord

Antagonists	Guinea-pig ileum		Gerbil spinal cord	
	pA_2	slope	pA_2	slope
Compound I	8.70 ± 0.06 (4)	0.90 ± 0.05 (4)	7.07 ± 0.18 (4)	0.93 ± 0.07 (4)
Compound II	7.56 ± 0.05 (5)	1.09 ± 0.04 (5)	5.93 ± 0.07 (4)	1.11 ± 0.05 (4)
Compound III	8.41 ± 0.05 (4)	0.98 ± 0.003(4)	6.40 ± 0.10 (4)	1.08 ± 0.11 (4)
CP-99,994	8.27 ± 0.06 (4)	0.93 ± 0.03 (4)	7.26 ± 0.03 (3)	1.02 ± 0.02 (3)

The pA_2 values were determined from Arunlakshana–Schild plots. Effects of the antagonists on the contractile response to substance P in the guinea-pig ileum was examined in the presence of atropine (4 μM), mepyramine (4 μM) and indomethacin (3.6 μM) and on the depolarizing action of substance P in the neonatal gerbil spinal cord in the presence of tetrodotoxin (0.3 μM). Each value is mean ± S.E.M. Number of determinations in parentheses.

99,994 at 0.01–0.1 μM caused parallel rightward shifts of the concentration–response curve of substance P in a concentration-dependent manner (Fig. 2g; Natsugari et al., 1995a). The Schild plot gave a slope of 0.93 ± 0.03 (n = 4), which was close to unity and the pA_2 value of CP-99,994 against substance P was 8.27 ± 0.06 (n = 4, Table 1). In contrast, CP-99,994 at 0.1 μM did not alter the concentration–response curve of acetylcholine in the guinea-pig ileum (Fig. 2h). CP-99,994 at concentrations of up to 0.1 μM did not induce contraction (data not shown). These results suggest that CP-99,994 is a specific and competitive antagonist on tachykinin NK_1 receptors in the guinea-pig ileum (McLean et al., 1993b).

Likewise, addition of compounds I, II and III caused parallel rightward shifts of the concentration–response curve of substance P in a concentration-dependent manner (Fig. 2a, c and e). The slopes of Schild plots were again close to unity and the pA_2 values of compounds I, II and III against substance P were 8.70, 7.56 and 8.41, respectively (Table 1). Addition of compounds I, II and III at 0.1–0.3 μM had no inhibitory action on the concentration–response curve of acetylcholine in the guinea-pig ileum (Fig. 2b, d and f) and compounds I, II and III did not induce contraction by themselves.

Effects of the antagonists on tachykinin NK_2 receptors were examined in the rat vas deferens, which is known to possess predominantly tachykinin NK_2 receptors (Regoli et al., 1988). Compounds I, II and III at 0.3, 0.3 and 0.1 μM, respectively, as well as CP-99,994 at 0.3 μM caused no shift of the concentration-response curve of neurokinin A in the rat vas deferens (Fig. 3).

In order to examine the actions of the tachykinin receptor antagonists on tachykinin NK_3 receptors, their effects on contraction of rat portal vein induced by senktide were observed (Wormser et al., 1986; Mastrangelo et al., 1987). Compounds I, II and III at 0.3, 0.3 and 0.1 μM, respectively, as well as CP-99,994 at 0.3 μM did not significantly inhibit the contraction induced by senktide at 10 nM (Fig. 4).

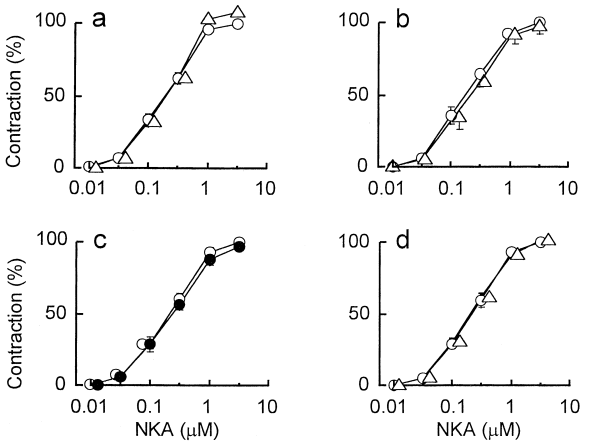

Fig. 3. Effects of compounds I, II, III and CP-99,994 on the concentration–response curve of neurokinin A (NKA) in the rat vas deferens. Ordinate: peak height of neurokinin A-induced contractile response expressed as percentages of maximal control response to neurokinin A. Abscissa: logarithmic molar concentration of neurokinin A. Each symbol and bar represent the mean ± S.E.M. of 3 to 5 observations. (a) Effect of compound I; (b) compound II; (c) compound III and (d) CP-99,994. Other details are the same as described for Fig. 2. ○, control; ● and △, after addition of tachykinin receptor antagonists at 0.1 and 0.3 μM, respectively.

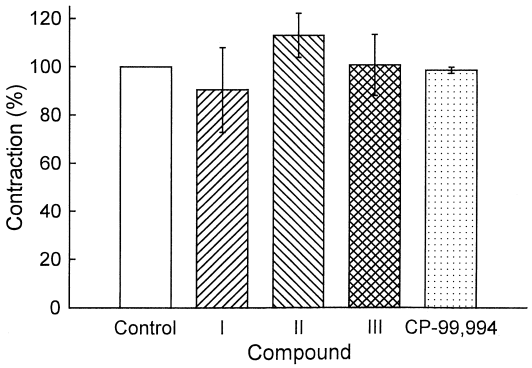

Fig. 4. Effects of compounds I, II, III and CP-99,994 on contractile response to senktide in the rat portal vein. Senktide was applied at 10 nM. Each column represents the peak height of senktide-induced contractile responses expressed as the percentage of the average of control responses. The open column represents control response, and upward hatched, downward hatched, crossed and dotted columns, after addition of compound I, II, III and CP-99,994 at 0.3, 0.3, 0.1 and 0.3 μM, respectively. Each column and vertical bar represents the mean ± S.E.M. of 3 observations.

These results altogether suggest that compounds I, II and III are specific and competitive antagonists of tachykinin NK_1 receptors in peripheral tissues.

3.2. Effects of compounds I, II, III and CP-99,994 on tachykinin receptors in the gerbil spinal cord

The effects of the antagonists on responses to substance P and L-glutamate of spinal motoneurons were examined after eliminating synaptic transmission by tetrodotoxin in the isolated gerbil spinal cord preparation (cf. Yanagisawa and Otsuka, 1990). Substance P at 0.03–10 μM evoked depolarizing responses of ventral roots in a concentration dependent manner and pD_2 of substance P was 6.27 ± 0.06 ($n = 4$, Fig. 5). Addition of CP-99,994 at 0.1–0.3 μM caused parallel rightward shifts of the concentration–response curve of substance P in a concentration-dependent manner (Fig. 5g). The Schild plot gave a slope of 1.02 ± 0.02 ($n = 3$) and pA_2 was 7.26 ± 0.03 ($n = 3$, Table 1). Similarly, compounds I, II and III at 1–10 μM caused parallel rightward shifts of the concentration–response curve of substance P in a concentration-dependent manner (Fig. 5a, c and e). The slopes of the Schild plot for compounds I, II and III against substance P were close to unity and the pA_2 values of compounds I, II and III against substance P were 7.07, 5.93 and 6.40, respectively (Table 1). In contrast, compounds I (3 μM), II (10 μM), III (3 μM) and CP-99,994 (1 μM) did not alter the concentration–response curve of L-glutamate (Fig. 5b, d, f and h). These results again suggest that compounds I, II and III as well as CP-99,994 are specific and competitive tachykinin NK_1 receptor antagonists in the neonatal gerbil spinal cord.

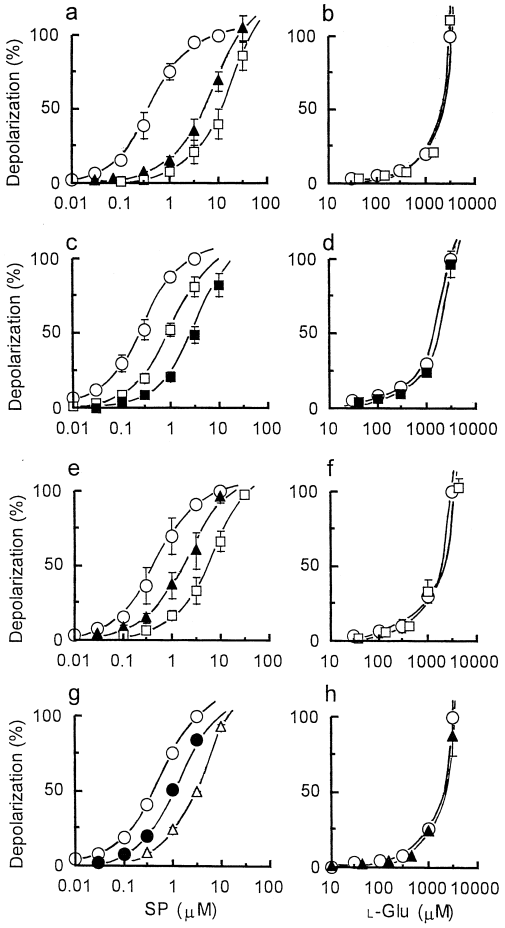

Fig. 5. Effects of compounds I, II, III and CP-99,994 on the concentration–response curves of substance P (SP) and L-glutamate (L-Glu) in the neonatal gerbil spinal cord in the presence of tetrodotoxin (0.3 μM). Ordinate: peak amplitude of agonist-induced depolarization of ventral roots expressed as percentage of control response to substance P (3–10 μM) or L-glutamate (3 mM) before adding these antagonists. Abscissa: logarithmic molar concentration of each agonist. Each symbol and bar represent the mean ± S.E.M. of 3 to 4 observations. For some symbols, standard errors were smaller than the symbols and therefore not shown. Some symbols are displaced horizontally to avoid overlapping. (a, c, e and g) Concentration–response curves of substance P and (b, d, f and h) concentration–response curves of L-glutamate. (a and b) Effects of compound I; (c and d) effects of compound II; (e and f) effects of compound III and (g and h) effects of CP-99,994. ○, control; ●, △, ▲, □ and ■, after addition of each tachykinin antagonist at 0.1, 0.3, 1, 3 and 10 μM, respectively.

4. Discussion

The present study showed that a series of amide-based derivatives, i.e. compounds I, II and III, act as specific tachykinin NK_1 receptor antagonists in both the peripheral tissue and the central nervous system of mammals, i.e. guinea-pig ileum and gerbil spinal cord. Previous studies on tachykinin NK_1 receptor antagonists revealed that mammalian species can be divided into two groups with respect to the characteristics of their tachykinin NK_1 receptors (Beresford et al., 1991; Fardin and Garret, 1991; Fardin et al., 1993). Some antagonists, such as CP-96,345 and CP-99,994, have higher affinities to tachykinin NK_1 receptors of one group of species including human, rabbit, guinea-pig, hamster and gerbil than those of another group of species including rodents (Beresford et al., 1991; Gitter et al., 1991; Snider et al., 1991; Barr and Watson, 1993; Fardin et al., 1993; McLean et al., 1993b). In contrast, another antagonist, RP 67580, has higher affinities to tachykinin NK_1 receptors of rodents than those of human, gerbil and guinea-pig (Barr and Watson, 1993; Fardin et al., 1993; Hosoki et al., 1994). Natsugari et al. (1995a) showed that compound I has a higher affinity to tachykinin NK_1 receptor of human IM-9 cell line (IC_{50}, 0.21 nM) than that to tachykinin NK_1 receptors of rat forebrain (IC_{50}, 0.43 μM). This result suggests that compound I, as well as compounds II and III, belongs to the former group of antagonists including CP-99,994.

The present study showed that compound I is more active than CP-99,994 in the guinea-pig ileum and slightly less active in the gerbil spinal cord. A remarkable characteristic of compound I is that it is orally much more active than CP-99,994. Natsugari et al. (1995a) reported that

compound I inhibited the capsaicin-induced plasma extravasation in the guinea-pig trachea with ED_{50} values of 0.017 mg/kg by i.v. administration and 0.068 mg/kg by oral administration. In contrast, CP-99,994 inhibited the plasma extravasation with ED_{50} values of 0.017 mg/kg by i.v. and 8.7 mg/kg by oral administrations. Since compound I is orally active and since it has a high affinity to human tachykinin NK_1 receptors (Natsugari et al., 1995a), the possibility of its clinical use may be worthy of exploration. Furthermore, compound I may serve as a lead compound for the future development of clinically useful tachykinin NK_1 receptor antagonists.

A tachykinin NK_1 receptor antagonist, CP-96,345 was shown to display a high affinity for [^{3}H]-diltiazem binding sites on L-type calcium channels (Guard et al., 1993). In this respect, Natsugari et al. (1995a) showed that compound I did not displace or enhance [^{3}H]-nitrendipine binding to membranes from rat brain cortices at the concentrations of 10 μM, suggesting the lack of interaction with L-type calcium channels.

The pA_2 values of compound I against substance P are 8.70 in the guinea-pig ileum and 7.07 in the gerbil spinal cord. Likewise the pA_2 values of CP-99,994 are 8.27 in the guinea-pig ileum and 7.26 in the gerbil spinal cord. The pA_2 values of CP-96,345, spantide, GR 71251 and GR 82334 against substance P in the guinea-pig ileum are 8.5, 6.28, 7.5 and 7.5, respectively, whereas, the pA_2 values of these antagonists against substance P in the gerbil spinal cord are 7.08, 5.82, 6.56 and 6.2, respectively (Beresford et al., 1991, 1992; Guo et al., 1993, 1995; Otsuka et al., 1994 and unpublished observations). Altogether, the pA_2 values of these antagonists against substance P are higher in the guinea-pig ileum than in the gerbil spinal cord. These differences of pA_2 values of the tachykinin NK_1 receptor antagonists in the guinea-pig ileum and the gerbil spinal cord may be partly due to the species difference, but may suggest a possible difference between tachykinin NK_1 receptors of peripheral tissues and central nervous system.

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