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European Journal of Pharmacology 281 (1995) 49–54

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Pharmacological characterization of GR82334, a tachykinin NK_1 receptor antagonist, in the isolated spinal cord of the neonatal rat

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Received 2 January 1995; revised 4 April 1995; accepted 6 April 1995

Abstract

Pharmacological characteristics of [D-Pro^9,[spiro-γ-lactam]Leu^{10},Trp^{11}]physalaemin-(1-11) (GR82334), a tachykinin NK_1 receptor antagonist, and its effects on slow depolarizing responses of lumbar ventral roots evoked by primary afferent stimulation were examined in isolated spinal cord preparations of neonatal rats. GR82334 (1–3 μM) caused dose-dependent rightward shifts of the concentration-response curves for substance P, substance P methyl ester, δ-aminovaleryl [Pro^9,*N*-Me-Leu^{10}]substance P-(7–11) (GR73632) and neurokinin A in normal artificial cerebrospinal fluid and those for substance P methyl ester, GR73632 and neurokinin A in the presence of tetrodotoxin. GR82334 (10 μM) did not evoke γ-aminobutyric acid (GABA) release from spinal cords of neonatal rats, whereas [D-Pro^9,[spiro-γ-lactam] Leu^{10},Trp^{11}]substance P (GR71251), another tachykinin NK_1 receptor antagonist, induced a significant increase in GABA release. GR82334 (1–3 μM) markedly depressed the slow depolarizing response of ventral roots, referred to as slow ventral root potential, evoked by stimulation of the contralateral dorsal root or the ipsilateral saphenous nerve. In contrast, cyclo[Gln,Trp,Phe,Gly,Leu,Met] (L-659,877, 1 μM), a selective tachykinin NK_2 receptor antagonist, did not depress the saphenous nerve-evoked slow ventral root potential and did not antagonize the action of neurokinin A to induce ventral root depolarization. The present results provide further evidence for the involvement of substance P, neurokinin A and tachykinin NK_1 receptors in the primary afferent-evoked slow ventral root potentials.

Keywords: Tachykinin receptor antagonist; GR82334; GR71251; L-659,877; Substance P; Neurokinin A

1. Introduction

We have previously reported that, in isolated spinal cord preparations of neonatal rats, [D-Pro^9,[spiro-γ-lactam]Leu^{10},Trp^{11}]substance P (GR71251), a tachykinin NK_1 receptor antagonist (Hagan et al., 1990), depresses the slow depolarizing response of lumbar ventral roots evoked by stimulation of the contralateral dorsal root or the ipsilateral saphenous nerve (Guo et al., 1993). The slow depolarizing response evoked by primary afferent stimulation will be referred to as slow ventral root potential in the present paper. The results with GR71251, together with other lines of evidence, suggested the involvement of tachykinins, substance P and neurokinin A, in the slow ventral root potentials (Yanagisawa et al., 1982; Akagi et al., 1985; Otsuka and Yanagisawa, 1988; Nussbaumer et al., 1989). Although GR71251 is a potent and highly specific tachykinin NK_1 receptor antagonist in the neonatal rat spinal cord (Guo et al., 1993), this compound evokes the release of histamine from mast cells (Hagan et al., 1990) and the release of γ-aminobutyric acid (GABA) from neonatal rat spinal cords (see below).

Hagan et al. (1991) recently reported on another tachykinin receptor antagonist, [D-Pro^9,[spiro-γ-lactam]Leu^{10},Trp^{11}]physalaemin-(1–11) (GR82334), which was shown to be selective for tachykinin NK_1 receptors in the ileum longitudinal muscle and tracheal spiral

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SSDI 0014-2999(95)00228-6

strip of the guinea pig. GR82334 differs from GR71251 in that its *N*-terminus is different from that of substance P but similar to that of physalaemin. As a result, GR82334 does not release histamine from mast cells (Hagan et al., 1991). In this study we examined the pharmacological profile of GR82334 in the neonatal rat spinal cord and its effects on the slow ventral root potentials evoked by stimulation of primary afferent fibres.

2. Materials and methods

2.1. Preparations

Isolated spinal cord and spinal cord-saphenous nerve preparations were used (Akagi et al., 1985; Nussbaumer et al., 1989; Guo et al., 1993). The spinal cord below thoracic segments with attached spinal nerves was isolated with or without hemisection from neonatal Wistar rats of either sex aged 1–3 days. The preparation was placed in a recording chamber of 0.5 ml volume and perfused with artificial cerebrospinal fluid (CSF) saturated with 95% O_2-5% CO_2 at a flow rate of 2–4 ml/min. The temperature of the chamber was kept at 27° C. The composition of the artificial CSF was as follows (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. In the experiments in which the effects of GR82334 on the actions of exogenous tachykinins and bombesin were examined in normal artificial CSF, the concentration of $MgCl_2$ was increased to 2 mM in order to depress spontaneous activity.

2.2. Electrophysiological experiments

Potentials were recorded extracellularly with a suction electrode from a ventral root (L3-L5) in the isolated hemisected or whole spinal cord, and from the L3 ventral root in the whole spinal cord-saphenous nerve preparation, and were led through a d.c. amplifier to a pen recorder and a computer recording device (Axotape). Tachykinin receptor agonists and other agonists were bath-applied for 30 s at intervals of 10–90 min to hemisected spinal cord, and the areas under the curves of depolarizations were determined in mV · s. To examine the specificity of GR82334 the effects of the antagonist on actions of various agonists at the concentrations close to the EC_{70} values were determined. The effect of L-659,877 on the depolarizing action of neurokinin A was also determined at a concentration close to the EC_{70} of neurokinin A. The effects of these antagonists were evaluated after treatment of spinal cords with GR82334 for at least 5 min and with L-659,877 for at least 10 min.

To estimate pA_2 values of GR82334 against tachykinin receptor agonists, the concentration-response curve for each agonist was constructed first in normal or tetrodotoxin-containing artificial CSF and then after equilibration of the preparation with GR82334 for 5 min at two increasing concentrations. The antagonist-induced displacement of the concentration-response curve for each agonist was quantified as the ratio of equipotent molar concentrations at the half-maximum response level of the control concentration-response curve and the pA_2 value was determined from the Arunlakshana-Schild plot (Arunlakshana and Schild, 1959).

To evoke slow ventral root potentials the contralateral dorsal root of the same segment or ipsilateral saphenous nerve was stimulated with 2–5 shocks (40–50 V intensity, 100 μs duration at 20 Hz) every 90–120 s. The average areas (mV · s) under the curves of two to three consecutive responses were measured before and after adding GR82334 or L-659,877, and expressed as percentages of the control value obtained just before adding GR82334 or L-659,877.

2.3. Release experiments

GABA release from neonatal rat spinal cords was measured as described previously (Sakuma et al., 1991). Briefly, a hemisected spinal cord was perfused in a 1-ml chamber at a rate of 0.7 ml/min with artificial CSF. 3-min fractions of the perfusate were collected and the GABA contents were determined using high performance liquid chromatography.

2.4. Statistical analysis

The values were expressed as means ± S.E.M. The statistical significance of differences between means was evaluated with Student's *t*-test. Differences were considered significant when $P < 0.05$.

2.5. Drugs

The drugs used and their sources were as follows: GR71251 ([D-Pro^9,[spiro-γ-lactam]Leu^{10},Trp^{11}]substance P), GR73632 (δ-aminovaleryl [Pro^9,*N*-Me-Leu^{10}]substance P-(7–11)), GR82334 ([D-Pro^9,[spiro-γ-lactam]Leu^{10},Trp^{11}]physalaemin-(1–11)) (Glaxo Group Research); substance P, neurokinin A, substance P methyl ester, bombesin and thyrotropin-releasing hormone (Peptide Institute, Osaka, Japan); L-659,877 (cyclo[Gln,Trp,Phe,Gly,Leu,Met], Cambridge Research Biochemicals); senktide (succinyl-[Asp^6,Me-Phe^8]substance P-(6–11); Wormser et al., 1986) was a gift from Prof. Z. Selinger, Department of Biological Chemistry, the Hebrew University of Jerusalem, Israel. Other drugs were obtained from various commercial sources.

3. Results

3.1. Effects of GR82334 on tachykinin-evoked depolarizations

Bath applications of the tachykinin receptor agonists, substance P, substance P methyl ester, GR73632 and neurokinin A, to neonatal rat spinal cords both in normal and tetrodotoxin-containing artificial CSF produced concentration-dependent depolarizations of lumbar ventral roots. It is to be noted that the main site of action of tachykinin agonists and antagonists in the absence of tetrodotoxin is probably spinal interneurones and that in the presence of tetrodotoxin is motoneurones (Yanagisawa and Otsuka, 1990; Hosoki et al., 1994). GR82334 (1–3 μM) caused rightward shifts of the concentration-depolarization curves for substance P, substance P methyl ester, GR73632 and neurokinin A in normal artificial CSF and those for substance P methyl ester, GR73632 and neurokinin A in the presence of tetrodotoxin (0.3 μM). About 20 min after washing out GR82334, the responses to these agonists recovered to the original levels. Table 1 shows the pA_2 values of GR82334 against substance P, substance P methyl ester, GR73632 and neurokinin A in normal artificial CSF and in the presence of tetrodotoxin. There were slight but statistically significant differences between these values: the pA_2 value of GR82334 against substance P in normal artificial CSF (6.24) was lower than those against substance P methyl ester (6.68), GR73632 (7.09) and neurokinin A (6.56) in normal artificial CSF; and the pA_2 value against substance P methyl ester in the presence of tetrodotoxin (6.33) was lower than those against GR73632 (6.86) and neurokinin A (6.83) in the presence of tetrodotoxin. The slopes of Schild plots for GR82334 against these agonists were close to unity (1.01–1.12) suggesting competitive antagonism. On the other hand, the action of GR82334 (1–3 μM) against substance P in the presence of tetrodotoxin was complex, i.e., the antagonist depressed the depolarizations induced by low concentrations of substance P (0.3–1 μM) but slightly potentiated those induced by high concentrations of substance P (3–10 μM) (data not shown, cf. Yanagisawa and Otsuka, 1990).

Table 1
pA_2 values and slopes of the Schild plot for GR82334 against actions of tachykinin agonists to evoke ventral root depolarization in normal artificial CSF and in the presence of tetrodotoxin

Agonist	pA_2	Slope	n
In normal artificial CSF			
Substance P	6.24 ± 0.04	1.01 ± 0.06	3
Substance P methyl ester	6.68 ± 0.05 [a,c]	1.07 ± 0.04	3
GR73632	7.09 ± 0.07 [b,d]	1.09 ± 0.05	3
Neurokinin A	6.56 ± 0.04 [a,c]	1.01 ± 0.01	3
In the presence of tetrodotoxin			
Substance P methyl ester	6.33 ± 0.06	1.00 ± 0.01	3
GR73632	6.86 ± 0.08 [a,d]	1.12 ± 0.05	3
Neurokinin A	6.83 ± 0.01 [b,e]	1.01 ± 0.04	4

Each agonist was applied for 30 s and the area under the curve of the depolarization was measured in mV·s. The pA_2 values were determined from Arunlakshana-Schild plots of the concentration-response curves in the absence and presence of GR82334. The data are expressed as means ± S.E.M. [a] $P < 0.01$ and [b] $P < 0.001$, when compared with the pA_2 value against substance P in normal artificial CSF. [c] $P < 0.05$, [d] $P < 0.01$ and [e] $P < 0.001$, when compared with the pA_2 value against substance P methyl ester in the presence of tetrodotoxin.

Table 2
Effects of GR82334 (3 μM) and L-659,877 (1 μM) on ventral root depolarizing actions of various agonists in the neonatal rat spinal cord

Antagonist	Agonist	% of control
GR82334	Neurokinin A (3 μM)	8.4 ± 0.9 [c]
	Acetylcholine (2 mM)	93.9 ± 1.0 [b]
	L-Glutamate (3 mM)	95.7 ± 5.3
	GABA (1 mM)	95.3 ± 5.6
	Norepinephrine (5 μM)	121.7 ± 5.0 [a]
	Thyrotropin-releasing hormone (0.4 μM)	119.7 ± 7.8
	Senktide (1 μM)	97.6 ± 5.7
	Bombesin (70 nM)	99.5 ± 5.1
L-659,877	Neurokinin A (3 μM)	99.1 ± 2.1

The area under the curve of the depolarizing response to each agonist in mV·s was measured and expressed as percentage of its control value. Each agonist was applied for 30 s at a concentration close to the EC_{70}. The actions of senktide and bombesin were examined in normal artificial CSF whereas the actions of other agonists were examined in the presence of tetrodotoxin (0.3 μM). The data are expressed as means ± S.E.M. ($n = 3$–4). [a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$ compared with 100%.

3.2. Specificity of GR82334

The effects of GR82334 on the responses to senktide and bombesin in normal artificial CSF and those to other agonists in the presence of tetrodotoxin were examined with agonist concentrations close to EC_{70} (Table 2). GR82334 at 3 μM, which depressed the response to neurokinin A (3 μM) to 8.4% of the control, did not alter those to L-glutamate (3 mM), GABA (1 mM), thyrotropin-releasing hormone (0.4 μM), senktide (0.3 μM) and bombesin (70 nM). The antagonist slightly potentiated the response to norepinephrine (5 μM) and slightly depressed that to acetylcholine (2 mM).

3.3. Effect of L-659,877 on neurokinin A-evoked depolarization

Fig. 1 and Table 2 show the effects of L-659,877, a selective tachykinin NK_2 receptor antagonist (McKnight et al., 1991), on the response to neurokinin A in the presence of tetrodotoxin. L-659,877, which depressed the eledoisin-mediated response in rat vas

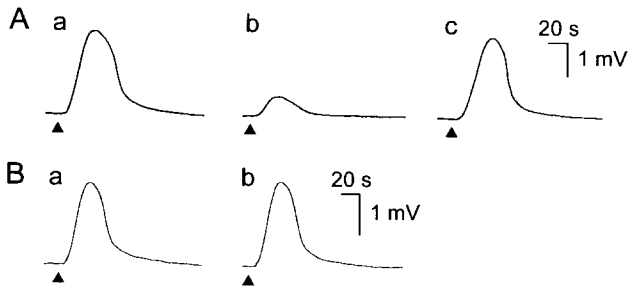

Fig. 1. Comparison of the effects of GR82334 and L-659,877 on the depolarization of ventral roots induced by neurokinin A in the presence of tetrodotoxin. neurokinin A (3 μM) was bath-applied for 30 s at (▲) every 10–15 min. (A) **a**, control response, **b**, 8 min after adding GR82334 (1 μM), and **c**, 20 min after washing out GR82334. (B) **a**, control response, and **b**, 30 min after adding L-659,877 (1 μM). Records in (A) and (B) are from different preparations.

deferens with a pA_2 value of 8.1 (McKnight et al., 1991), did not alter the neurokinin A (3 μM)-evoked depolarization at 1 μM, whereas GR82334 at 1 μM potently depressed the neurokinin A-evoked response.

3.4. Effects of GR82334 and L-659,877 on primary afferent-evoked slow ventral root potentials

Fig. 2 and Table 3 illustrate the effects of GR82334 and L-659,877 on the slow ventral root potentials

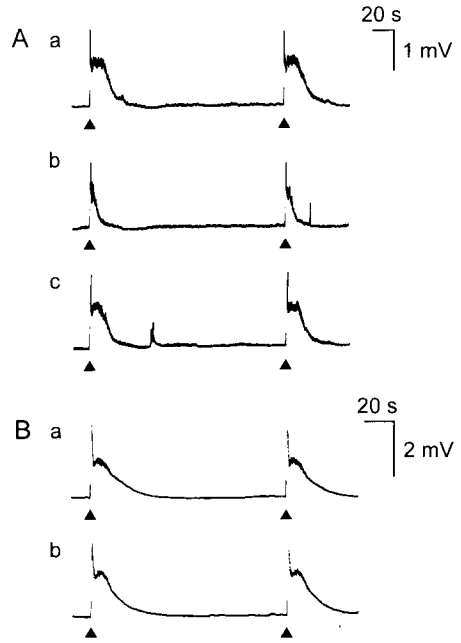

Fig. 2. Comparison of the effects of GR82334 and L-659,877 on the ipsilateral saphenous nerve-evoked slow ventral root potential. (A) **a**, control response, **b**, 15 min after adding GR82334 (3 μM), and **c**, 30 min after washing out GR82334. (B) **a**, control response, and **b**, 10 min after adding L-659,877 (1 μM). The saphenous nerve was stimulated with two shocks at 20 Hz every 180 s in (A) and every 120 s in (B), respectively. Records in (A) and (B) are from different preparations.

Table 3
Effects of GR82334 and L-659,877 on primary afferent-evoked slow ventral root potentials

Antagonist		Contralateral dorsal root-evoked slow ventral root potential		Ipsilateral saphenous nerve-evoked slow ventral root potential	
GR82334	1 μM	58.4±2.6 [b]	($n=5$)	44.1±3.0 [a]	($n=3$)
	3 μM	50.2±2.9 [b,c]	($n=5$)	43.5±3.0 [a]	($n=3$)
L-659,877	1 μM			108.4±9.4	($n=3$)

The area under the curve of the slow ventral root potential in mV·s was measured and expressed as percentage of the control value. The data are expressed as means±S.E.M. [a] $P<0.01$, and [b] $P<0.001$, when compared with 100 %. [c] $P<0.05$, when compared with the corresponding value under GR82334 at 1 μM by paired t-test.

evoked by stimulation of the contralateral dorsal root and the ipsilateral saphenous nerve. GR82334 at 1 and 3 μM dose dependently depressed the contralateral dorsal root-evoked slow ventral root potential to 58.4% and 50.2% of the control, respectively. The antagonist at 1 and 3 μM also potently depressed the ipsilateral saphenous nerve-evoked slow ventral root potential to 44.1% and 43.5%, respectively. The slow ventral root potentials recovered almost completely in 10–20 min after wash-out of GR82334. In contrast, L-659,877 at 1 μM did not show a significant depressant action on the ipsilateral saphenous nerve-evoked slow ventral root potential.

3.5. Effects of GR82334 on GABA release

GR82334 (10 μM) did not significantly increase GABA release from spinal cords, whereas GR71251 (10 μM), as well as substance P (10 μM), caused an increase in GABA release (Fig. 3).

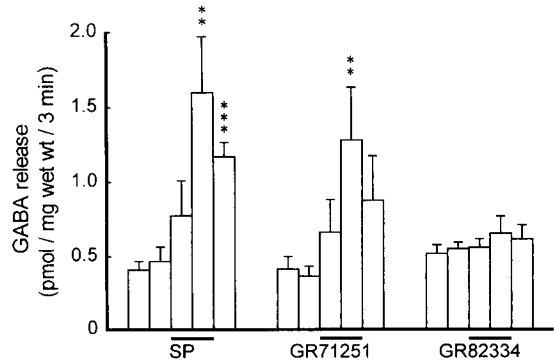

Fig. 3. Effects of substance P, GR71251 and GR82334 on the GABA release from isolated spinal cords. In each experiment five successive 3-min fractions were collected. Each agent was applied at 10 μM by perfusion for 6 min during the period indicated by the horizontal bar. The data are expressed as means±S.E.M ($n=3$). ** $P<0.01$ and *** $P<0.001$, when compared with the pre-control value (average of fractions 1 and 2).

4. Discussion

Our previous study showed that substance P evokes the release of GABA from neonatal rat spinal cords and that the action has characteristics similar to those of substance P action to evoke the release of histamine from mast cells (Sakuma et al., 1991). Since spantide and GR71251 possess similar GABA releasing actions (Sakuma et al., 1991 and the present study), it is conceivable that the depressant effects of these tachykinin antagonists on primary afferent-evoked slow ventral root potentials (Nussbaumer et al., 1989; Guo et al., 1993) might be due to the release of GABA or some other neurotransmitters (Surprenant et al., 1987). In the present study, however, GR82334 was found to be devoid of the GABA releasing action and the antagonist still depressed the slow ventral root potentials evoked by stimulation of the dorsal root and saphenous nerve to about 50% of the control. Since this maximal depressant effect of GR82334 was similar to those of spantide and GR71251 (Guo et al., 1993), the present results suggest that the tachykinin antagonists depress the slow ventral root potentials by their action on tachykinin receptors but not through the GABA-releasing action.

GR82334 potently depressed the depolarizing response of ventral roots to the tachykinin NK_1-selective agonists, substance P methyl ester and GR73632, in a competitive manner, which is consistent with its pharmacological profile as a tachykinin NK_1-selective antagonist in peripheral tissues (Hagan et al., 1991). However, the pA_2 values of GR82334 against substance P in normal artificial CSF (6.24) and against substance P methyl ester in the presence of tetrodotoxin (6.33) were slightly but significantly lower than the other pA_2 values obtained in the absence or presence of tetrodotoxin (6.56–7.09) as listed in Table 1. Similar differences between the potencies of antagonist actions against various tachykinin agonists were previously observed for spantide and GR71251 in the neonatal rat spinal cord (Guo et al., 1993; Yanagisawa and Otsuka, 1990) and for GR82334 in the rat superior cervical ganglion (Hawcock et al., 1993), suggesting the existence of more than one subtype of tachykinin NK_1 receptors.

The ventral root depolarizing action of neurokinin A was antagonized by GR82334, but not by L-659,877. Previous studies from our laboratory showed that other tachykinin NK_1 receptor antagonists, i.e. spantide, GR71251 and RP 67580, depressed the neurokinin A-evoked responses both in normal and in tetrodotoxin-containing artificial CSF (Yanagisawa and Otsuka, 1990; Guo et al., 1993; Hosoki et al., 1994) and that selective tachykinin NK_2 receptor agonists, [β-Ala^8]neurokinin A-(4–10), [Nle^{10}]neurokinin A-(4–10) (Guo et al., 1993) and GR64349 (unpublished data), evoked little depolarization of ventral roots. These results suggest that neurokinin A evokes ventral root depolarization through tachykinin NK_1 but not NK_2 receptors in the spinal cord of the neonatal rat. This notion is consistent with the finding that tachykinin NK_2 receptor mRNA is undetectable or at a very low level in the rat spinal cord (Tsuchida et al., 1990; Suzuki et al., 1993).

The slow ventral root potentials evoked by primary afferent stimulation were depressed by GR82334, but not by L-659,877. These results further support the involvement of tachykinins, substance P and neurokinin A, and tachykinin NK_1 receptors in these responses (Guo et al., 1993; Hosoki et al., 1994).

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (Nos. 04255101, 05557117 and 05454147).

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