Involvement of the Central Tachykinin NK₁ Receptor during Maintenance of Mechanical Hypersensitivity Induced by Diabetes in the Rat

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ABSTRACT

Our study examines the role of central and peripheral neurokinin, (NK₁) receptors in diabetes-induced mechanical hypersensitivity. Glycine, N, N-dimethyl-, 2-[[2-[[2-benzofuranylmethoxy]carbonyl][amino]-3-(1H-indol-3-yl)-2-methyl-1-oxopropyl]amino]-2-phenylethylester, bisulfate, [R-(R*,R*)] (PD 156982) is a selective NK₁ receptor antagonist with nanomolar affinity for the human (IC₅₀ = 1.4 nM) and guinea pig (IC₅₀ = 9.6 nM) NK₁ receptors. However, it has approximately two orders of magnitude lower affinity for the rodent NK₁ receptor (IC₅₀ = 820 nM). In electrophysiological studies, PD 156982 inhibited NK₁ receptor-mediated responses in the guinea pig locus ceruleus, in a competitive manner, with an equilibrium constant of 13.9 nM. The intracerebroventricular (10-100 mg/kg, s.c.) blocked the [Sar⁹,Met(O²)¹¹] substance P-induced gerbil foot tapping response. This indicates that PD 156982 is unable to penetrate into the central nervous system. However, PD 156982 (10-100 mg/kg, s.c.) blocked the mechanical hypersensitivity induced by administration of substance P into the plantar surface of a rat paw. This suggests that PD 156982 can effectively antagonize peripheral NK₁ receptors in vivo. The chemically related compound carbamic acid, [1-(1H-indol-3-ylmethyl)-1-methyl-2-oxo-2-[1-phenylethyl][amino][ethyl]-, 2-benzofuranylmethyl ester, [R-(R*,S*)] (CI-1021) is also a selective NK₁ receptor antagonist but can penetrate into the central nervous system. PD 156982 (10-100 mg/kg, s.c.) failed to block streptozocin (75 mg/kg, i.p.) induced mechanical hypersensitivity. In contrast, CI-1021 dose-dependently (3-100 mg/kg, s.c.) blocked this hypersensitivity state with a minimum effective dose of 10 mg/kg. At these doses CI-1021 also antagonized mechanical hypersensitivity mediated by central NK₁, but not NK₂ receptors in the rat. It is suggested that the central NK₁ receptor may play an important role in diabetes-induced hypersensitivity.

Substance P has long been known to be an important neurotransmitter implicated in the transmission of nociception (Henry, 1980). It is a member of the tachykinin family of neuropeptides that include neurokinin A and neurokinin B. Substance P is co-localized with neurokinin A in small diameter, high threshold nociceptive C-fibers and is released in response to a variety of noxious stimuli (Otsuka and Yoshioka, 1993). Three subclasses of G-protein coupled tachykinin receptors have been described in the CNS and periphery: NK₁, NK₂ and NK₃ receptors. However, it has approximately two orders of magnitude lower affinity for the rodent NK₁ receptor (IC₅₀ = 820 nM). In electrophysiological studies, PD 156982 inhibited NK₁ receptor-mediated responses in the guinea pig locus ceruleus, in a competitive manner, with an equilibrium constant of 13.9 nM. The intracerebroventricular (10-100 mg/kg, s.c.) blocked the [Sar⁹,Met(O²)¹¹] substance P-induced gerbil foot tapping response. This indicates that PD 156982 is unable to penetrate into the central nervous system. However, PD 156982 (10-100 mg/kg, s.c.) blocked the mechanical hypersensitivity induced by administration of substance P into the plantar surface of a rat paw. This suggests that PD 156982 can effectively antagonize peripheral NK₁ receptors in vivo. The chemically related compound carbamic acid, [1-(1H-indol-3-ylmethyl)-1-methyl-2-oxo-2-[1-phenylethyl][amino][ethyl]-, 2-benzofuranylmethyl ester, [R-(R*,S*)] (CI-1021) is also a selective NK₁ receptor antagonist but can penetrate into the central nervous system. PD 156982 (10-100 mg/kg, s.c.) failed to block streptozocin (75 mg/kg, i.p.) induced mechanical hypersensitivity. In contrast, CI-1021 dose-dependently (3-100 mg/kg, s.c.) blocked this hypersensitivity state with a minimum effective dose of 10 mg/kg. At these doses CI-1021 also antagonized mechanical hypersensitivity mediated by central NK₁, but not NK₂ receptors in the rat. It is suggested that the central NK₁ receptor may play an important role in diabetes-induced hypersensitivity.

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ABBREVIATIONS: PWT, paw withdrawal threshold; MED, minimum effective dose; NK, neurokinin; ACSF, artificial cerebrospinal fluid; NSAIDS, nonsteroidal antiinflammatory drugs; i.c.v. intracerebroventricular. CI-1021, carbamic acid, [1-(1H-indol-3-ylmethyl)-1-methyl-2-oxo-2-[1-phenylethyl][amino][ethyl]-, 2-benzofuranylmethyl ester, [R-(R*,S*)]; PD 156982, glycine, N,N-dimethyl-2-[2-[[2-benzofuranylmethoxy]carbonyl][amino]-3-(1H-indol-3-yl)-2-methylloxopropyl][amino]-2 phenylethyl ester, bisulfate, [R-(R*,R*)].
have limited therapeutic value in the management of diabetes-induced neuropathic pain (Galer, 1995; James and Page, 1994). This has led to the use of adjuvant analogies for the management of this condition. At present tricyclic antidepressants are currently the first choice in the treatment of painful diabetic neuropathy (Galer, 1995; James and Page, 1994). However, no agent is fully effective in all patients and undesirable side effects are common (Galer, 1995; James and Page, 1994). Streptozocin is a selective pancreatic β-cell toxin that has been used to induce experimental diabetes in laboratory animals (Tomlinson et al., 1992). The resultant loss of endogenous insulin induced by streptozocin mimics the characteristics of type I or insulin-dependent diabetes. Streptozocin-induced diabetes has recently been described as a model of chronic pain. It has been reported that streptozocin administration leads to mechanical, thermal and chemical hyperalgesia as well as mechanical hypersensitivity detected using von Frey hairs (Courteix et al., 1993a; Calcutt et al., 1996). The mechanical hyperalgesia induced by streptozocin is sensitive to tricyclic antidepressants, clonidine and lidocaine treatment (Courteix et al., 1994). However, it is less sensitive to aspirin and morphine (Courteix et al., 1994). Thus, these profiles mimic their respective clinical activities observed in the treatment of diabetic neuropathy. The most common symptoms of diabetic neuropathy appear to be spontaneous burning pain and mechanical hypersensitivity (where normally innocuous tactile stimuli induce pain) in the feet or lower limbs. Our study investigates the role of peripheral and central NK1 receptors in the maintenance of diabetes-induced mechanical hypersensitivity in the rat.

Methods

Mongolian gerbils (40-70 g) of either sex (Bantin and Kingman, Hull, UK) were housed in groups of eight. Male Sprague Dawley rats (200-250 or 250-300 g), obtained from Bantin and Kingman were housed in groups of six. Male Dunkin-Hartley guinea pigs (350-400 g) were obtained from Interfauna (Huntingdon, UK). All animals were kept under a 12-hr light/dark cycle (lights on at 07 hr 00 min) with food and water ad libitum. All experiments were carried out by an observer blind to drug treatments.

Receptor Binding Assays

Tachykinin receptor binding assays. Tachykinin NK1 receptor binding assays were carried out as described previously (Boyle et al., 1994). Human lymphoma IM9 cells were grown in RPMI 1640 culture medium supplemented with 10% fetal calf serum and 2 mM glutamine and maintained under an atmosphere of 5% CO2. Cells were harvested for experiments by centrifugation at 1000 × g for 3 min. Pelleted cells were washed once by resuspension into assay buffer (50 mM Tris HCl pH 7.4, 3 mM NaN3, 0.02% bovine serum albumin, 40 μg/ml bacitracin, 2 μg/ml chymostatin, 2 μM phosphoramidon, 4 μg/ml leupeptin) and repeating the centrifugation step before resuspending at a concentration of 2.5 × 10^6 cells/ml assay buffer. Cells (200 μl) were incubated with [125I]Bolton-Hunter substance P. Reactions were terminated by rapid filtration under vacuum onto GFC filters presoaked in 0.2% polyethylene imine for 1 to 2 hr, using a Brandel cell harvester. Filters were washed with 6 × 1 ml ice-cold Tris HCl (50 mM, pH 7.4) and bound radioactivity determined using a gamma counter. Results were analyzed using iterative curve fitting procedures in RS1 (BBN Software Products, Cambridge, MA) or Graphpad Inplot. Binding affinity data were expressed as the geometric mean and range of IC_{50} values determined from three to six separate experiments unless otherwise indicated.

For tachykinin NK1 receptor binding assays using membranes prepared from brain tissues, P2 tissue was prepared by initially homogenizing brain tissue in 10 vols 0.32 M sucrose and centrifuging at 1000 × g for 10 min. The supernatant was recentrifuged at 40,000 × g for 15 min, the pellet washed twice by resuspension and centrifugation before resuspending at a concentration appropriate for adding 2 to 5 mg wet weight of P2 pellet per tube, depending on the species.

Binding assays for tachykinin NK2 receptors were carried out as described previously (Guard et al., 1993) using [125I]NK A and membranes prepared from hamster urinary bladder.

Tachykinin NK3 receptor binding assays were carried out as described previously (Suman-Chauhan et al., 1994) using [125I] [MePhe7]NK1 B and membranes prepared from Chinese hamster ovary -K1 cells expressing cloned human NK3 receptors.

Selectivity assays. To determine selectivity, 23 binding assays for non tachykinin receptors or binding sites were carried out using standard methodology as described in the literature. The species and radioligands used are shown in table 1.

Electrophysiology

Coronal slices of brainstem 350-μm thick containing the locus ceruleus were cut from the brains of guinea pigs. One such slice was placed in a Perspex recording chamber where it was pinned down on to a Sylgard base. The slice was placed in a perfusion chamber and perfused with ACSF flowing at 4 ml/min. The ACSF contained (in mM) NaCl 126; KCl 5; NaH2PO4 1.2; MgCl2 1.3; CaCl2 2.4; NaHCO3 26 and glucose 10. It was gassed with a carbogen mixture (95% oxygen and 5% carbon dioxide). All experiments were performed at 37°C. Extracellular recordings were made from spontaneously firing individual neurones in the locus ceruleus using low resistance (5-10 Mohm when filled with ACSF) glass microelectrodes. Details of the subsequent data analysis have been reported elsewhere (Boden et al., 1991). The agonist, in this case [Sar8, Met(O2)11] substance P, was applied for 1 min. Slices were pretreated with antagonists for 30 min before addition of agonist in the continued presence of the antagonist.

Induction of [Sar9, Met(O2)11] Substance P-Induced Foot Tapping

Gerbils were briefly anesthetized with an isoflurane O2/NO2 mixture. An incision was made into the scalp to expose the skull. [Sar8, Met(O2)11] substance P was administered i.c.v. in a volume of 5 μl by vertical insertion of a cuffed 27-gauge needle to a depth of 4.5 mm below bregma. Animals were placed individually into observation boxes and duration of hind paw tapping was recorded for 5 min immediately after recovery of the animals righting reflex. For antagonism studies PD 156982 (1-100 mg/kg) was administered or i.c.v. (10-100 μg/animal) 0.25 h before the injection of [Sar8, Met(O2)11] substance P.

Effect of CI-1021 on Hypersensitivity Induced by Central Administration of Substance P or [βAla8]NK A (A-4-10)

Rats (200-250 g) were administered with substance P or [βAla8]NK A (A-4-10) i.t., in a volume of 10 μl using a 100 μl Hamilton syringe, by exposing the spine under brief isoflurane O2/NO2 anesthesia. Injections of agonists were made into the i.t. space between lumbar region 5-6 with a 10 mm long 27-gauge needle, with penetrations being judged successful by a tail flick response. The wound was sealed with an autoclip and rats appeared fully awake within 2 to 3 min after injection. Baseline PWT to von Frey hairs were determined before drug treatment. CI-1021 (formally PD 154075) or its vehicle was administered s.c. 30 min before i.t. substance P (8

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μg/animal) or [βAla8]NK A(4-10) (0.2 μg/animal). PWT were determined again at various times up to 1 hr post i.t. injection.

**Effect of PD 156982 on Hypersensitivity Induced by Administration of Substance P into the Hind Paw of Rats**

Rats (200-250 g) were administered with substance P in a volume of 50 μl into the plantar surface of the left hind paw using a 100 μl Hamilton syringe. Baseline PWT, using von Frey hairs were determined before drug treatment. PD 156982 or its vehicle was administered s.c. 30 min before intraplantar substance P (5 μg/animal). PWT were determined again at 10 and 20 min post i.t. injection.

**Development of Diabetes in the Rat**

Diabetes was induced in 40 rats (250-300 g) by a single i.p. injection of streptozocin (75 mg/kg) as described previously (Courteix et al., 1993a). Control animals (n = 8) received a similar administration of isotonic saline.

**Effect of CI-1021 and PD 156982 on the Maintenance of Streptozocin-Induced Mechanical Hypersensitivity**

CI-1021 was examined between 11 to 12 days post streptozocin. PD 156982 was examined in the same set of animals between 14 to 15 days post streptozocin. On each test day baseline PWT to von Frey hairs were determined before drug treatment. CI-1021 (3-100 mg/kg), PD 156982 (10-100 mg/kg) or their vehicle was administered s.c. and PWT reexamined every hour for up to 5 hr.

**Evaluation of Mechanical Hypersensitivity**

Mechanical hypersensitivity was measured using Semmes-Weinstein von Frey hairs (Stoelting, Wood Dale, IL). Animals were placed into wire mesh bottom cages allowing access to the underside of their paws. Animals were habituated to this environment before the start of the experiment. Mechanical hypersensitivity was tested by touching the plantar surface of the animals hind paw with von Frey hairs in ascending order of force (0.7, 1.2, 1.5, 2, 3.6, 5.5, 8.5, 11.8, 15.1 and 29 g) for up to 6 sec. Once a withdrawal response was established, the paw was retested, starting with the next descending von Frey hair until no response occurred. The highest force of 29 g lifted the paw as well as eliciting a response, thus represented the cut-off point. The lowest amount of force required to elicit a response was recorded as the PWT in grams.

**Drugs Used**

CI-1021 and PD 156982 were synthesized at Parke-Davis Neuroscience Research Centre (Cambridge, UK). Both compounds were dissolved in polyethylene glycol-200 for systemic administration. For i.c.v. administration PD 156982 was dissolved in hydroxypropyl-β-cyclodextrin (20% in saline). [Sar8, Met(O2)11] substance P (Sigma, Poole, UK) was dissolved in saline with 0.01% acetic acid. Streptozocin (Aidrich, Gillingham, UK), substance P (Sigma, Poole, UK) and [βAla8]NK A(4-10) (RBI, Natick, MA) were dissolved in 0.9% w/v NaCl. Systemic drug administrations were made in a volume of 2 ml/kg in gerbils and 1 ml/kg in rats. The i.e.v. i.t. and intraplantar administrations were made in respective volumes of 5, 10 and 50 μl.

**Statistics**

Data obtained from the foot tapping model were subjected to unpaired Student t test comparing to the relevant vehicle-treated group. The mechanical hypersensitivity results obtained using the von Frey hairs were subjected to an individual Mann Whitney U test and compared to vehicle-treated animals.

**Results**

Radioligand binding studies. PD 156982 exhibited high affinity for tachykinin NK1 receptors present in human lymphoma IM9 cells with an IC50 value (mean with minimum and maximum range shown in parentheses) of 1.4 nM (0.4-4.3). PD 156982 also possessed high affinity for NK1 receptors in the guinea pig, dog, ferret and hamster, with IC50 values of 9.6 (8.1-14), 9.6 (4.2-19), 21 (10-45) and 10 nM (5.2-36), respectively. In contrast, affinities of PD 156982 for rodent NK1 receptors in the rat and mouse were at least two orders of magnitude lower than human receptors [IC50 values 820 nM (630-1050) and 530 nM (340-820) respectively].

PD 156982 exhibited high selectivity for human NK1 over NK2 receptors in hamster urinary bladder (IC50 value > 10 μM) and cloned human NK2 receptors expressed in CHO cells [IC50 value 2460 nM (640-4030)]. Furthermore, a high degree of selectivity was also observed against a wide range of non-tachykinin receptors, with PD 156982 showing < 50% inhibition of specific binding at 10 μM in the majority of assays (table 1). Of the exceptions amongst the 23 assays screened, highest affinity was observed at CCKA receptors in the rat pancreas [IC50 value 450 nM (range 270-600)]. IC50 values in the 2 to 10 μM range were obtained for cloned human BB1 and BB2 receptors, CCKA and 5-HT2 receptors, and calcium channels labeled by [3H]-nimodipine (table 1).

**Electrophysiology.** The locus ceruleus preparation contains functional NK1 receptors which are excited in a concentration-dependent manner by the selective NK1 agonist [Sar8 Met(O2)11] substance P. PD 156982 (300 nM) blocked the response to [Sar8 Met(O2)11] substance P in an agonist surmountable manner (fig. 1a), indicative of competitive antagonism, yielding an equilibrium constant (Kc) of 13.9 nM (fig. 1b). Kc values were within the range 13.9 to 36 nM for the series of experiments, in accord with the binding affinity for PD 156982 at the guinea pig and human NK1 receptor.

**Effect of PD 156982 on hypersensitivity induced by administration of substance P into the hind paw of diabetic rats.**

**Table 1**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholecystokinin CCK A</td>
<td>[125I]BHCCK8s</td>
<td>Rat pancreas</td>
</tr>
<tr>
<td>Cholecystokinin CCK B</td>
<td>[125I]BHCCK8s</td>
<td>Cloned human</td>
</tr>
<tr>
<td>BB1</td>
<td>[125I]Tyr</td>
<td>bombesin</td>
</tr>
<tr>
<td>BB2</td>
<td>[125I]Tyr</td>
<td>bombesin</td>
</tr>
<tr>
<td>Galanin</td>
<td>[125I]Galalin</td>
<td>Rat basal forebrain</td>
</tr>
<tr>
<td>Opiate σ</td>
<td>[3H]U69593</td>
<td>Guinea pig forebrain</td>
</tr>
<tr>
<td>Opiate μ</td>
<td>[3H]DIDAGOL</td>
<td>Guinea pig forebrain</td>
</tr>
<tr>
<td>Na/K ATPase</td>
<td>[3H]Ouabain</td>
<td>Rat cortex</td>
</tr>
<tr>
<td>Dopamine D 1</td>
<td>[3H]SCH23390</td>
<td>Pig striatum</td>
</tr>
<tr>
<td>5-HT</td>
<td>[3H]5HT</td>
<td>Guinea pig cortex</td>
</tr>
<tr>
<td>5-HT-αA</td>
<td>[3H]5OH-DPAT</td>
<td>Guinea pig cortex</td>
</tr>
<tr>
<td>5-HTβ</td>
<td>[3H]Retanoserin</td>
<td>Rat cortex</td>
</tr>
<tr>
<td>K+ channel ATP sensitive</td>
<td>[3H]Gibencalmine</td>
<td>Pig brain</td>
</tr>
<tr>
<td>Benzodiazepine</td>
<td>[3H]Flunitrazepam</td>
<td>Mouse brain</td>
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<tr>
<td>L-type Ca++ channel</td>
<td>[3H]Nimodipine</td>
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<td>Histamine H1</td>
<td>[3H]Pyrilamin</td>
<td>Pig cortex</td>
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<td>Adrenoceptor α-1</td>
<td>[3H]Prazosin</td>
<td>Rat cortex</td>
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<tr>
<td>muscarinic M1</td>
<td>[3H]Hqnb</td>
<td>Pig brain</td>
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<td>GABA-A</td>
<td>[3H]Gabab</td>
<td>Rat cortex</td>
</tr>
<tr>
<td>GABA-B</td>
<td>[3H]Gabab</td>
<td>Rat cortex</td>
</tr>
<tr>
<td>Glycine</td>
<td>[3H]Glucine</td>
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<tr>
<td>NMDA</td>
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</tr>
<tr>
<td>NMDA</td>
<td>[3H]GCP3963</td>
<td>Rat cortex</td>
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</tbody>
</table>

List of nontachykinin receptor binding assays performed to determine selectivity of PD 156982 for tachykinin NK1 receptors. Assays were carried out using standard methodology as described in the literature. PD 156982 showed <50% inhibition of specific binding at 10 μM in the majority of assays. Of the exceptions, highest affinity was observed at CCKB receptors in the rat pancreas [IC50 value 450 nM (range 270-600)]. IC50 values in the 2 to 10 μM range were obtained for human BB1 and BB2 receptors, CCKA and 5-HT2 receptors, and calcium channels labeled by [3H]-nimodipine.
nmol/animal of [Sar⁹, Met⁷(O₂)¹¹] substance P was chosen for antagonism studies as it represents a submaximal dose (Singh et al., 1997). The systemic administration of PD 156982 (1-100 mg/kg, s.c.), failed to block the [Sar⁹, Met⁷(O₂)¹¹] substance P-induced foot tapping response at any pretreatment time (fig. 2a). However, when PD 156982 was administered i.c.v. 15 min before [Sar⁹, Met⁷(O₂)¹¹] substance P, it dose-dependently (10-100 μg/animal) antagonized the foot tapping response, with a MED of < 10 μg/animal (fig. 2b).

Effect of CI-1021 on hypersensitivity induced by central administration of substance P or [βAla⁸]NK A(4-10). The i.t. administration of substance P or [βAla⁸]NK A(4-10) induced mechanical hypersensitivity which was present post injection for up to 40 and 60 min respectively (fig. 3). Pretreatment with CI-1021 dose-dependently (10-100 mg/kg, s.c.) blocked the development of substance P-induced hypersensitivity with a MED of 30 mg/kg (fig. 3a). The top dose of 100 mg/kg completely blocked the development of mechanical hypersensitivity (fig. 3a). In contrast, similar administration of 100 mg/kg CI-1021 failed to show any block of the [βAla⁸]NK A(4-10)-induced hypersensitivity (fig. 3b).

Effect of CI-1021 and PD 156982 on diabetes-induced mechanical hypersensitivity. The single injection of streptozocin (75 mg/kg, i.p.) induced mechanical hypersensitivity which developed fully within 11 days post treatment. The administration of CI-1021 dose-dependently (3-100 mg/kg, s.c.) blocked the maintenance of this hypersensitivity with a MED of 10 mg/kg (fig. 4a). The dose of 100 mg/kg completely antagonized the mechanical hypersensitivity, with PWT similar to those of nondiabetic control animals (fig. 4a). The antihypersensitive action of 100 mg/kg CI-1021 was apparent for approximately 4 hr. In contrast, similar administration of PD 156982 (10-100 mg/kg, s.c.) failed to block the hypersensitivity exhibited by streptozocin animals (fig. 4b).

Discussion

PD 156982: a peripherally selective NK₁ receptor antagonist. The radioligand binding results presented here show that PD 156982 possesses nanomolar affinity for human and guinea pig NK₁ receptors. However, as with many other NK₁ receptor antagonists, it has approximately two orders of magnitude lower affinity for the rodent NK₁ receptor. This confirms previous reports that guinea pig and human tachykinin NK₁ receptors are similar to each other but different to rodent NK₁ receptors (Maggi, 1994). PD 156982 displays a high degree of selectivity for the human type NK₁ receptor over NK₂ and NK₃ receptor types. The electrophys-

![Fig. 1. Effect of PD 156982 on the [Sar⁹, Met⁷(O₂)¹¹] Substance P-induced increase in firing rate of a locus ceruleus neurone.](image)

TABLE 2

<table>
<thead>
<tr>
<th>Time Post Substance P (min)</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL</td>
<td>Median</td>
<td>First</td>
</tr>
<tr>
<td>Veh.</td>
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</tr>
<tr>
<td>10</td>
<td>11.75</td>
<td>11.75</td>
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<tr>
<td>30</td>
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<td>11.75</td>
</tr>
<tr>
<td>100</td>
<td>11.75</td>
<td>11.75</td>
</tr>
</tbody>
</table>

Mechanical hypersensitivity was assessed using von Frey hairs. Baseline (BL) PWT to von Frey hair filaments were determined before drug treatment. PD 156982 (mg/kg) or its vehicle was administered s.c. 30 min prior to intraplantar substance P (8 μg/animal). PWT were determined again at various times post intraplantar injection. Results are expressed as median force (g) required to induce a withdrawal in 7 to 10 animals per group (with first and third quartile range).

* P < .001, ** P < .005, significantly different from vehicle (Veh.) treated animals (Mann Whitney U test).
iological data indicate that PD 156982 is a competitive antagonist at central NK 1 receptors in the guinea pig. It was found to be inactive in most binding assays, suggesting that it is a selective NK1 receptor antagonist. Previously, it has been shown that central activation of NK 1 receptors induces a characteristic foot tapping response in the gerbil (Graham et al. 1993; Bristow and Young, 1994; Rupniak and Williams, 1994). The antagonism of this response by systemic administration of NK1 receptor antagonists has been suggested to reflect their ability to penetrate into the brain (Graham et al., 1993; Bristow and Young, 1994; Rupniak and Williams, 1994). Thus, the failure of PD 156982 to block the foot tapping after systemic administration indicates that the drug cannot penetrate into the CNS.

**Effect of peripherally selective and CNS penetrating NK1 receptor antagonists on diabetes-induced mechanical hypersensitivity.** CI-1021 is a selective NK 1 receptor antagonist, but unlike PD 156982, it can penetrate into the CNS (Singh et al., 1997). Our results show that systemic administration of CI-1021 can block the maintenance of diabetes-induced mechanical hypersensitivity. This is consistent with a recent study showing that the rat selective but chemically unrelated NK1 receptor antagonist RP-67580 can reduce diabetes-induced mechanical hyperalgesia (Courteix et al., 1993b). Our results further show that PD 156982 can block mechanical hypersensitivity mediated by peripheral NK1 receptors in the rat. This indicates that in vivo, PD 156982 is an effective antagonist at peripheral NK1 receptors. Thus, its failure to block diabetes-induced hypersensitivity highlights the importance of the central NK1 receptor in the maintenance of this state of hypersensitivity.

It is known that CI-1021 shows species differences with respect to its affinity for the NK1 receptor (Singh et al., 1997). Thus, it possesses at least two orders of magnitude higher affinity for the human and guinea pig than the rodent NK1 receptor. This low affinity for the rodent NK1 receptor may account for the high doses that were required in the present study to block diabetes-induced hypersensitivity in the rat. It is possible that at these high doses CI-1021 loses selectivity and the anti-hypersensitivity action does not involve the NK1 receptor. However, results of the previous and present study, appear to argue in favor of involvement of the NK1 receptor. Thus, our data show that CI-1021 can dose-dependently block hypersensitivity induced by activation of central NK1 receptors in the rat. Moreover, this antagonism was complete.

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Fig. 2. Effect of PD 156982 on [Sar9, Met(O2)11] substance P-induced foot tapping in the gerbil. PD 156982 was administered either (a) s.c. at various pretreatment times before, or (b) centrally at 0.25 hr before 30 nmol [Sar9, Met(O2)11] substance P (i.c.v). The duration of foot tapping was recorded for 5 min immediately after recovery from anesthesia. Vehicle treatments were administered for each separate time point in (a) and remained consistent throughout. For clarity only the vehicle response for the first time point is shown. Results are shown as the mean (vertical bars show S.E.M.) of 5 to 10 animals per group. *P < .05, **P < .01, Significantly different from the vehicle (Veh.) treated control group (unpaired Student t test).

Fig. 3. Effect of CI-1021 on the development of mechanical hypersensitivity induced by central administration of either (a) substance P or (b) [Ala8]NK A(4-10). Baseline (BL) PWT to von Frey hairs were determined before drug treatment. CI-1021 or its vehicle was administered s.c. 30 min before i.t. substance P (8 µg/animal) or [Ala8]NK A(4-10) (0.2 µg/animal). Results are expressed as median force (g) required to induce a withdrawal in 8-10 animals per group (vertical bars represent first and third quartiles). *P < .05, **P < .01, ***P < .005 significantly different from ipsilateral paw of agonist + vehicle (Veh.) treated animals (Mann Whitney U test).
Previously, radioligand binding studies have shown that CI-1021 may be acting selectively at the NK1 receptor as it had no effect on the NK2 receptor-mediated hypersensitivity. Furthermore, radioligand binding studies have shown that CI-1021 is a highly selective NK1 receptor antagonist (Singh et al., 1997). Taken together, these observations suggest that the NK1 receptor is likely to be the predominant site of action mediating the antihypersensitivity effect of CI-1021.

It is unclear whether the hypersensitivity state observed in our study is mediated by nociceptive Aδ/C-fibers or sensory Aβ-fibers. The low threshold, wide diameter Aβ-fibers are activated by innocuous stimulation. However, stimulation of these fibers in neuropathic conditions or during prolonged inflammation induces a noxious response (Devor, 1996). Recently, it has been shown that Aδ-fibers can synthesize and release substance P during prolonged inflammation (Neumann et al., 1996). This biochemical change may account for the induction of hypersensitivity in prolonged inflammation. However, the role of substance P in diabetic neuropathy seems to be unclear. It has been reported that substance P-like immunoreactivity is decreased in the sciatic nerve and spinal cord of streptozocin-induced diabetic rats (Brewster et al., 1994; Kamei et al., 1991). It has been shown that potassium can evoke an excessive release of substance P-like immunoreactivity from the spinal cord of diabetic rats (Kamei et al., 1991). It remains to be determined whether this increase in the release of substance P involves Aβ-fibers. Other studies have shown that in diabetic rats there is an up-regulation of substance P binding sites in the spinal cord (Kamei et al., 1990). The sensitization of dorsal horn neurones during chronic pain is induced by enhanced activity of C-fibers that release glutamate and substance P. The possible release of the neuropeptide from Aβ-fibers in diabetic neuropathy may contribute to the maintenance of spinal hypersensitivity. However, regardless of its origin the central action of substance P appears to be important for the maintenance of streptozocin-induced hypersensitivity. This explains the antihypersensitivity action of CI-1021 and the lack of effect of PD 156982, as blockade of central NK1 receptors would be necessary for this activity.

As with other neuropathic pain syndromes, conventional analgesics have limited therapeutic value in the treatment of painful diabetic neuropathy (Arné and Meyer, 1988; Galer, 1995; James and Page, 1994). Currently, there is no single effective treatment for the management of this painful syndrome and a number of adjuvant analgesics are used in combination (Galer, 1995; James and Page, 1994). They include tricyclic antidepressants and anticonvulsants. However, such treatments are limited by their physiological and psychological side effect profiles (Galer, 1995; James and Page, 1994). Our study demonstrates that a centrally acting NK1 receptor antagonist may possess utility for the treatment of painful diabetes-induced neuropathy. CI-1021 possesses higher affinity for the human compared with the rat NK1 receptor (Singh et al., 1997), therefore it is possible that much lower doses may show efficacy in man. However, it remains to be seen whether blocking part of the nociceptive transmission carried by substance P is sufficient, as in rats, to block painful diabetes-induced neuropathy in man.

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**References**


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