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Roles of mast cells and sensory nerves in cutaneous vascular hyperpermeability and scratching behavior induced by poly-L-arginine in rats

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Abstract

We investigated whether the polycation poly-L-arginine elicited cutaneous vascular hyperpermeability and scratching behavior and, if so, whether these responses involved mast cells and sensory nerves in rats. Intradermal injections of poly-L-arginine induced vascular hyperpermeability and scratching behavior. Combined treatment with chlorpheniramine and methysergide almost completely suppressed the poly-L-arginine (50 μg/site)-induced plasma leakage. Capsaicin desensitization and the *tachykinin* NK_1 *receptor* antagonist LY303870, (*R*)-1-[*N*-(2-methoxybenzyl)acetylamino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl)amino]propane, partially inhibited the leakage. In mast cell-deficient rats, poly-L-arginine only minimally induced plasma leakage. On the other hand, capsaicin desensitization and LY303870, but not chlorpheniramine or methysergide, suppressed the poly-L-arginine (200 μg/site)-induced scratching. Moreover, poly-L-arginine elicited the scratching even in mast cell-deficient rats. These results suggest that substance P is at least partly involved in both the cutaneous plasma leakage and the scratching behavior induced by poly-L-arginine. Moreover, mast cell-derived amines are suggested to be involved in the plasma extravasation but scarcely, if any, in the scratching behavior.

Keywords: Poly-L-arginine; Cutaneous vascular hyperpermeability; Scratching behavior; Mast cell; Sensory nerve; (Rat)

1. Introduction

In the patients with pruritic allergic dermatitis, the deposition of major basic protein (MBP), which is one of the eosinophil granule proteins, is frequently observed around the sites of skin lesions (Peters et al., 1983; Leiferman, 1989). Moreover, MBP, when intradermally injected into human skin, elicits a dose-related wheal-and-flare reaction (Leiferman et al., 1984). These findings suggest that MBP plays a crucial role in the pathogenesis of allergic cutaneous diseases.

MBP is a highly cationic polypeptide containing 17 arginine residues (Wasmoen et al., 1988). The previous study demonstrated that MBP induced airway hyperresponsiveness, which was inhibited by anionically charged heparin (Coyle et al., 1993). This observation suggests that the MBP-induced airway hyperresponsiveness depends on the cationic nature of this protein (Coyle et al., 1993; Uchida et al., 1993), and thus the synthetic cationic polypeptides have been used as tools to study the biological properties of MBP. In fact, the synthetic cationic polypeptides poly-L-lysine and poly-L-arginine are reported to elicit airway hyperresponsiveness in rats (Coyle et al., 1993; Uchida et al., 1993). Thus, the synthetic polycations are supposed to mimic MBP in the light of proinflammatory effect. In the skin, poly-L-lysine and poly-L-arginine increase cutaneous vascular permeability in rats (Stein et al., 1956) and rabbits (Needham et al., 1988), and poly-L-arginine induces wheal and flare in human skin (Foreman et al., 1983).

Histamine (Hägermark, 1974), serotonin (Weisshaar et al., 1997) and substance P (Hägermark et al., 1978) are known to induce flare and itch in human skin. Poly-L-lysine, poly-L-arginine and MBP induce histamine release

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from human skin mast cells (Benyon et al., 1989) and rat peritoneal mast cells (O'Donnell et al., 1983), while MBP and poly-L-lysine induce substance P release from cultured rat dorsal root ganglia (Garland et al., 1997). These observations suggest that MBP and the synthetic polycations may induce skin inflammation by the activation of mast cells to release amines and/or sensory C-fibers to release substance P.

In this article, we report that poly-L-arginine induces not only cutaneous vascular hyperpermeability but also scratching behavior in rats. As far as we know, there has been no report showing that MBP or the synthetic polycations induces itch-associated scratching behavior in animals, or elicits itching in humans. In this study, in order to investigate the possible roles of mast cells and sensory nerves in the cutaneous vascular hyperpermeability and the scratching behavior induced by poly-L-arginine, we employed mast cell-deficient rats and capsaicin desensitized rats and, moreover, determined the effects of antagonists for histamine H_1, *5-HT and tachykinin* NK_1 *receptors* on these responses.

2. Materials and methods

2.1. Animals

All animals were purchased from Japan SLC (Shizuoka, Japan). For the measurement of vascular permeability, 8- to 16-week-old male Wistar rats weighing 194–334 g, male mast cell-deficient rats (WsRC/Slc-Ws/Ws; 12 weeks of age, 253–303 g) and the control ones (WsRC/Slc-+/+; 12 weeks of age, 347–387 g) were used. For the observation of scratching behavior, 4- to 6-week-old male Wistar rats weighing 85–140 g, male mast cell-deficient rats (WsRC/Slc-Ws/Ws; 7 weeks of age, 159–205 g) and the control ones (WsRC/Slc-+/+; 7 weeks of age, 186–225 g) were used. The animals were acclimatized in an animal room maintained at a room temperature of 19–25 °C and a relative humidity of 30–70% with a 12-h light–dark cycle (illuminated between 07:00 and 19:00 h). Food and water were freely available. This animal experiment was approved by the Animal Ethical Committee of Kyowa Hakko Kogyo (Shizuoka, Japan).

2.2. Drugs

Poly-L-arginine hydrochloride (poly-L-arginine; MW 11,800 or 12,100, Sigma, St. Louis, MO, USA), poly-L-aspartic acid sodium salt (poly-L-aspartic acid; MW 11,100, Sigma), phosphoramidon sodium salt (phosphoramidon; Sigma), naloxone hydrochloride (naloxone; Sigma), serotonin creatinin sulfate (serotonin; Sigma), substance P (Peptide Institute, Osaka, Japan) and histamine dihydrochloride (histamine; Wako, Osaka, Japan) were dissolved in saline. Chlorpheniramine maleate (chlorpheniramine; Sigma) and methysergide maleate (methysergide; Janssen Pharmaceutica, Beerse, Belgium) were dissolved in distilled water. LY303870, (*R*)-1-[*N*-(2-methoxybenzyl) acetylamino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-(piperidin-1-yl) piperidin-1-yl)acetyl)amino]propane (synthesized at Kyowa Hakko Kogyo) was dissolved in acidified water, and the pH was adjusted to neutrality with 1 mol/l NaOH. Capsaicin (Wako) was dissolved in saline containing 10% ethanol and 10% Tween 80.

2.3. Capsaicin desensitization

Capsaicin desensitization was performed according to a modification of the method described by Del Monte et al. (1990). Increasing doses of capsaicin were injected for a total of 52 mg/kg (s.c.) over 2 days. On the first day, the doses of 0.3, 0.6, 1.2 and 2.4 mg/kg were injected at 2-h intervals. On the second day, 2.5, 10, 15 and 20 mg/kg were injected. Since the first five capsaicin injections are highly irritating, pentobarbital sodium was injected at the dose of 20 mg/kg (i.p.) 15 min before the first and the fifth injection of capsaicin, and at the dose of 10 mg/kg (i.p.) 15 min before the second, third and fourth injection of capsaicin. The animals were used 5 or 6 days after the final injection of capsaicin. In order to check the validity of this procedure, each rat received topical application of capsaicin (500 μg/ear) to the ear 1 day or 2 days before the intradermal injection of poly-L-arginine. Capsaicin did not induce ear oedema in any of desensitized rats tested.

2.4. Vascular permeability

The back skin of the rat was clipped. In the Wistar rat, poly-L-arginine (5–500 μg/site), histamine (5 μg/site), serotonin (0.5 μg/site) or substance P (5 ng/site) was injected intradermally at a volume of 50 μl/site. Immediately after the injection, the rat received 0.5 ml of 1% Evans blue (Wako) intravenously. The rat was sacrificed with CO_2 gas 30 min after the elicitation of the cutaneous reactions, and the skin at the reaction site was excised. The dye content in the skin specimen was measured according to a modification of the method described by Katayama et al. (1978). The specimen was dissolved in 1 ml of 1 mol/l KOH solution. Nine milliliters of a mixture of 0.6 mol/l H_3PO_4 solution and acetone (5:13) was added, and the sample was centrifuged at $1200 \times g$ for 10 min. The amount of dye extracted in the supernatant was measured colorimetrically at 620 nm (U-2001, Hitachi, Tokyo, Japan). The leaked dye amount was calculated by subtracting the dye content of untreated site from that of the intradermally injected site. To examine the possible involvement of cations in the poly-L-arginine-induced hyperpermeability, poly-L-aspartic acid was administrated intra-

dermally together with poly-L-arginine at a volume of 50 μl/site. In another series of the experiments, chlorpheniramine or methysergide was orally administrated at a volume of 10 ml/kg 1 h before the intradermal injection of histamine, serotonin or poly-L-arginine. Moreover, to examine the possible involvement of substance P, the neutral endopeptidase inhibitor phosphoramidon (Hashimoto et al., 1997) or the *tachykinin NK_1 receptor* antagonist LY303870 (Iyengar et al., 1997) was intraveneously injected at a volume of 1 ml/kg 5 min before the intradermal injection of substance P or poly-L-arginine.

To examine the possible role of mast cells, we employed the mast cell-deficient rats. In the mast cell-deficient (WsRC/Slc-Ws/Ws) rat or the control (WsRC/Slc-+/+) rat, poly-L-arginine (50 μg/site) was injected intradermally at a volume of 50 μl/site. The vascular permeability was determined as mentioned above.

2.5. Scratching behavior

Scratching behavior was observed according to a modification of the method described by Andoh et al. (1998). The rostral part of the back skin of the rat was clipped, and the rat was put into an acrylic cage composed of four cells (the size of each cell: 200 × 145 × 170 mm) and acclimated for about 30 min. In the Wistar rat, poly-L-arginine (50–200 μg/site), histamine (0.005–500 μg/site) (Inagaki et al., 1999; Yamaguchi et al., 1999), serotonin (0.005–500 μg/site) (Inagaki et al., 1999; Yamaguchi et al., 1999) or substance P (0.005–300 μg/site) (Andoh et al., 1998) was intradermally injected into the rostral part of the back (around the interscapular level) at a volume of 50 μl/site. Immediately thereafter, the rat was put back to the same cell, and the behavior was recorded using an 8-mm video camera (CCD-TRV95 NTSC, Sony, Tokyo, Japan) for 30 min. The scratching around the injected site with the hind-paws was counted. The rat generally scratched several times in about 1 s and a series of the scratchings was counted as one incidence. To examine the possible involvement of cations in the poly-L-arginine-induced scratching, poly-L-aspartic acid was administrated intradermally together with poly-L-arginine at a volume of 50 μl/site. Naloxone (Pini et al., 1997) was intraperitoneally injected at a volume of 1 ml/kg 15 min before the intradermal injection of poly-L-arginine. Chlorpheniramine or methysergide was orally administrated at a volume of 10 ml/kg 1 h before the intradermal injection of serotonin or poly-L-arginine. Moreover, to examine the possible involvement of substance P, phosphoramidon or LY303870 was intraveneously injected at a volume of 1 ml/kg 5 min before the intradermal injection of poly-L-arginine.

To examine the possible involvement of mast cells, we employed the mast cell-deficient rats. In the mast cell-deficient (WsRC/Slc-Ws/Ws) rat or the control (WsRC/Slc-+/+) rat, poly-L-arginine (200 μg/site) was intradermally injected into the rostral part of the back at a volume of 50 μl/site. The scratching behavior was examined as mentioned above.

2.6. Statistical analysis

The data were expressed as means ± standard error of the mean (S.E.M.). Analysis of statistical significance was performed with the SAS system for Windows ver. 6.12 (SAS Institute, Cary, NC, USA). In the experiment of vascular permeability, either the Student's *t*-test or the Aspin–Welch test was used for comparisons between the two groups, after the variances of the data were evaluated with the *F*-test. Multiple comparisons were made first by the one-way analysis of variance (ANOVA), followed by the Dunnett test when appropriate. In the experiment of scratching behavior, the Wilcoxon rank sum test was used for comparisons between the two groups. Multiple comparisons were made first by the Kruskal–Wallis test, followed by the Steel test when appropriate. Differences were considered significant if P values were < 0.05.

3. Results

3.1. Vascular hyperpermeability

In Wistar rats, an intradermal injection of poly-L-arginine (5, 50 and 500 μg/site) into the dorsal skin increased cutaneous vascular permeability with the increase of the injected dose. The Evans blue contents (μg/site) were 3.8 ± 0.64, 5.8 ± 0.45, 13.7 ± 1.59 and 29.8 ± 3.37 (mean ± S.E.M., $n = 7$) following 0, 5, 50 and 500 μg/site of poly-L-arginine, respectively. In the subse-

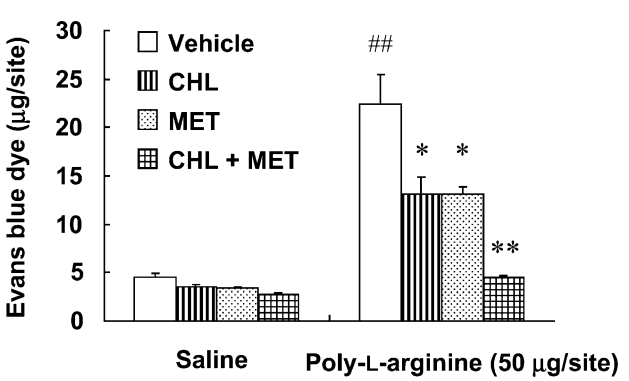

Fig. 1. Effects of chlorpheniramine (CHL, 10 mg/kg), methysergide (MET, 1 mg/kg) and chlorpheniramine (10 mg/kg) plus methysergide (1 mg/kg) (CHL + MET) on the vascular hyperpermeability induced by poly-L-arginine in the rat skin. Chlorpheniramine or methysergide was orally administered 1 h before the intradermal (i.d.) injection of saline or 50 μg/site of poly-L-arginine. Each value represents the mean ± S.E.M. from six animals. ##$P < 0.01$ compared with the value in the saline i.d. group (open column). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the value in the poly-L-arginine-injected control group.

quent experiments, 50 μg/site of poly-L-arginine was used since this dose produced the cutaneous vascular hyperpermeability sufficient to assess the effects of drugs.

Poly-L-aspartic acid (0.5, 5 and 50 μg/site, intradermally), a synthetic polyanion, inhibited the poly-L-arginine (50 μg/site)-induced increase of vascular permeability with the increase of the injected dose, and almost completely inhibited it at 50 μg/site. The Evans blue contents (μg/site) were 23.8 ± 1.35 for the control, 21.6 ± 1.30 at 0.5 μg/site, 17.9 ± 1.22 at 5 μg/site and 4.0 ± 0.51 at 50 μg/site (mean ± S.E.M., $n = 8$), respectively. Poly-L-aspartic acid at up to 50 μg/site did not affect vascular permeability by itself (data not shown).

Chlorpheniramine (10 and 20 mg/kg, p.o.), a histamine H_1 receptor antagonist, almost completely suppressed the histamine (5 μg/site)-induced cutaneous vascular hyperpermeability. The Evans blue contents (μg/site) were 22.9 ± 1.30 for the control, 3.6 ± 0.27 at 10 mg/kg and 3.0 ± 0.60 at 20 mg/kg (mean ± S.E.M., $n = 7$–8), respectively. Methysergide, a *5-HT receptor antagonist*, at 1 and 3 mg/kg almost completely suppressed the serotonin (0.5 μg/site)-induced cutaneous vascular hyperpermeability. The Evans blue contents (μg/site) were 24.4 ± 1.69 for the control, 11.3 ± 1.97 at 0.1 mg/kg, 4.3 ± 0.58 at 1

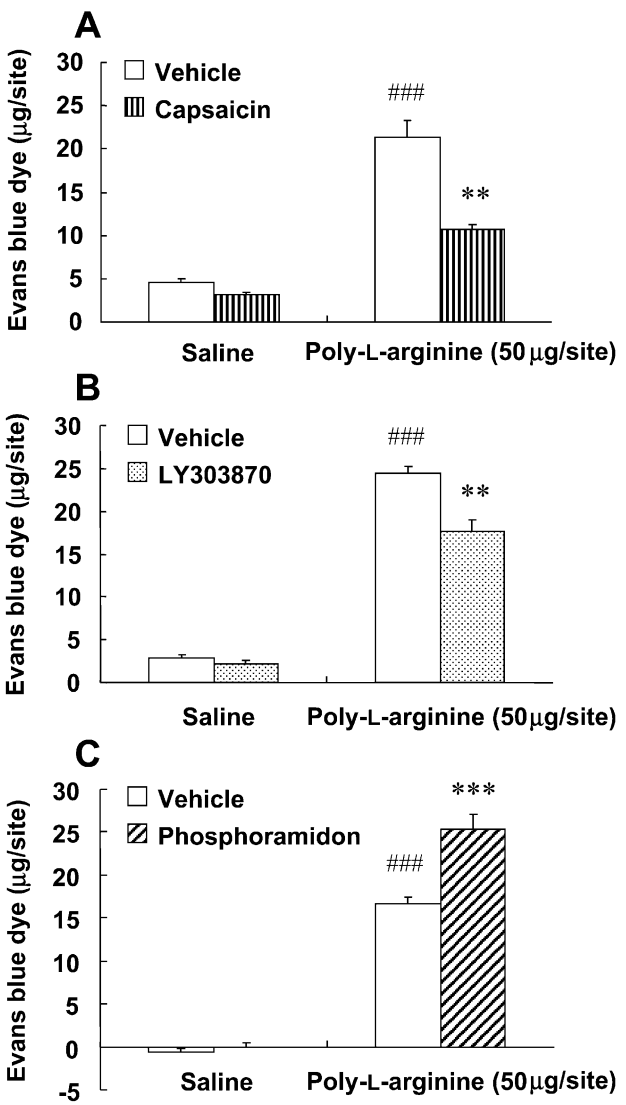

Fig. 2. Effects of capsaicin (52 mg/kg) (A), LY303870 (10 mg/kg) (B) and phosphoramidon (2.5 mg/kg) (C) on the vascular hyperpermeability induced by poly-L-arginine in the rat skin. Capsaicin was given subcutaneously in eight doses (0.3, 0.6, 1.2, 2.4, 2.5, 10, 15 and 20 mg/kg) over 2 days. The animals were used after 5 days of the last injection. LY303870 or phosphoramidon was administered intravenously 5 min before the intradermal (i.d.) injection of saline or 50 μg/site of poly-L-arginine. Each value represents the mean ± S.E.M. from 6 to 8 animals. $###P < 0.001$ compared with the value in the saline i.d. group (open column). $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the value in the poly-L-arginine-injected control group.

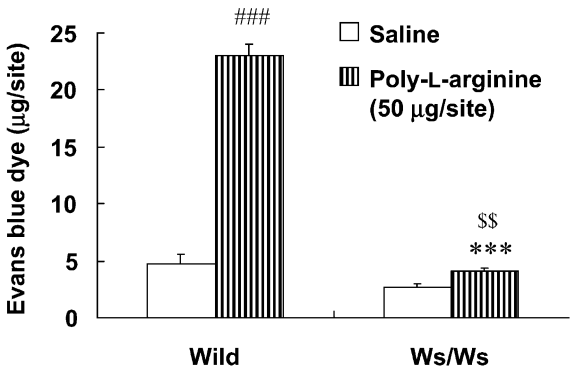

Fig. 3. Comparison of the poly-L-arginine-induced cutaneous plasma extravasation between the mast cell-deficient and the control rats. Poly-L-arginine (50 μg/site) was injected intradermally (i.d.) into the mast cell-deficient (Ws/Ws) rats and the control (Wild) ones. Each value represents the mean ± S.E.M. from six animals. $###P < 0.001$ compared with the saline i.d. group in control rats. $$$P < 0.01$ compared with the value in the saline i.d. group in mast cell-deficient rats. $^{***}P < 0.001$ compared with the value in the poly-L-arginine i.d. group in control rats.

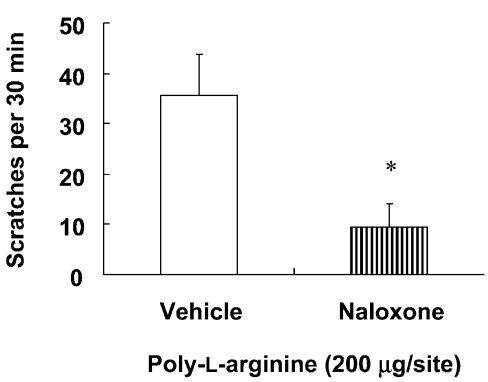

Fig. 4. Effects of naloxone (1 mg/kg) on the scratching behavior induced by poly-L-arginine. Saline or naloxone was administered intraperitoneally 15 min before the intradermal injection of 200 μg/site of poly-L-arginine. The scratching around the injected site with the hind-paws was counted for 30 min after the intradermal injection. Each value represents the mean ± S.E.M. from seven animals. $^{*}P < 0.05$ compared with the value in the vehicle-treated group.

mg/kg and 3.8 ± 0.32 at 3 mg/kg (mean ± S.E.M., $n = 5$), respectively. LY303870 (10 mg/kg, i.v.), a *tachykinin NK_1 receptor antagonist*, almost completely suppressed the substance P (5 ng/site)-induced cutaneous vascular hyperpermeability. The Evans blue contents (μg/site) were 25.5 ± 2.50 for the control and 5.3 ± 0.46 at 10 mg/kg (mean ± S.E.M., $n = 6$), respectively. Thereby, 10 mg/kg (p.o.) of chlorpheniramine, 1 mg/kg (p.o.) of methysergide and 10 mg/kg (i.v.) of LY303870 were used for assessing their effects on the poly-L-arginine-induced cutaneous vascular hyperpermeability.

Chlorpheniramine (10 mg/kg, p.o.) and methysergide (1 mg/kg, p.o.) inhibited the poly-L-arginine (50 μg/site)-induced vascular hyperpermeability by 46.4% and 45.8%, respectively, and their combination inhibited it by 90.5% (Fig. 1).

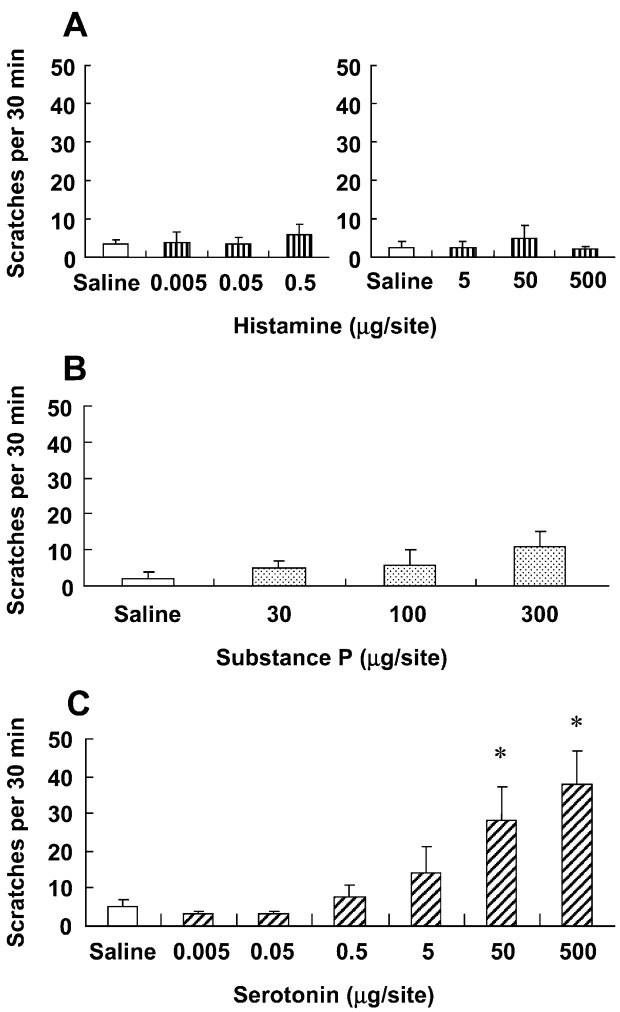

Fig. 5. Scratching behavior following intradermal injections of histamine (A), substance P (B) and serotonin (C). Histamine or serotonin at doses of 0.005–500 μg/site, or substance P at doses of 30–300 μg/site was injected intradermally into the rostral back of rats. The scratching around the injected site with the hind-paws was counted for 30 min after the injection. Each value represents the mean ± S.E.M. from six animals. * $P < 0.05$ compared with the value in the saline-injected group.

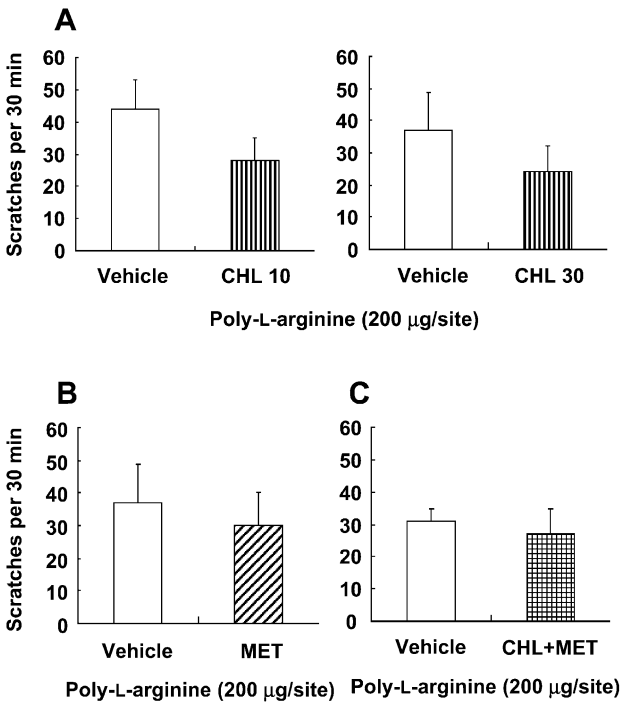

Fig. 6. Effects of chlorpheniramine (CHL, 10 and 30 mg/kg) (A), methysergide (MET, 3 mg/kg) (B) and chlorpheniramine (30 mg/kg) plus methysergide (3 mg/kg) (CHL + MET) (C) on the scratching behavior induced by poly-L-arginine. Chlorpheniramine or methysergide was orally administered 1 h before the intradermal injection of 200 μg/site of poly-L-arginine. The scratching around the injected site with the hind-paws was counted for 30 min after the intradermal injection. Each value represents the mean ± S.E.M. from eight animals.

Capsaicin desensitization (52 mg/kg, s.c.) and LY303870 (10 mg/kg, i.v.) inhibited the poly-L-arginine (50 μg/site)-induced vascular hyperpermeability by 54.8% and 28.7%, respectively (Fig. 2A and B). Phosphoramidon (2.5 mg/kg, i.v.), an inhibitor of neutral endopeptidase, enhanced the vascular hyperpermeability by 47.1% (Fig. 2C). Phosphoramidon (2.5 mg/kg, i.v.) did not affect vascular permeability by itself (Fig. 2C).

In mast cell-deficient (WsRC/Slc-Ws/Ws) rats, poly-L-arginine (50 μg/site, intradermally) induced minimal dye leakage, although it markedly increased vascular permeability in the control (WsRC/Slc-+/+) rats (Fig. 3). The dye leakage in WsRC/Slc-Ws/Ws rats was significantly smaller than that in the control rats (Fig. 3). No mast cells were histologically confirmed in the dorsal skin of WsRC/Slc-Ws/Ws rats (in 5 mm width: Ws/Ws, none; +/+, 117 ± 13 cells).

3.2. Scratching behavior

In Wistar rats, an intradermal injection of poly-L-arginine (50, 100 and 200 μg/site) into the rostral part of the back skin produced scratching behavior with the increase of the injected dose. The numbers of scratching

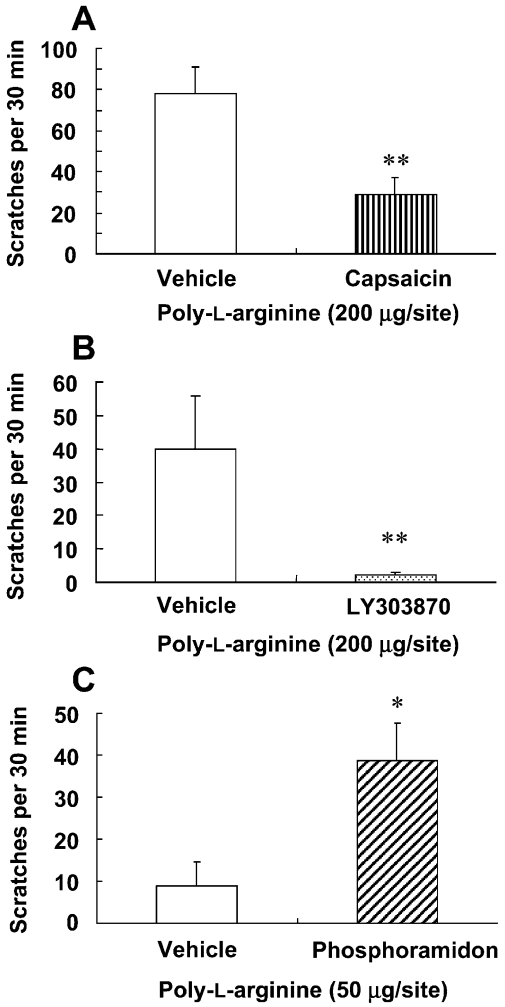

Fig. 7. Effects of capsaicin (52 mg/kg) (A), LY303870 (10 mg/kg) (B) and phosphoramidon (2.5 mg/kg) (C) on the scratching behavior induced by poly-L-arginine. Capsaicin was given subcutaneously in eight doses (0.3, 0.6, 1.2, 2.4, 2.5, 10, 15 and 20 mg/kg) over 2 days. The animals were intradermally injected with 200 μg/site of poly-L-arginine 6 days after the last injection of capsaicin. LY303870 or phosphoramidon was administered intravenously 5 min before the intradermal injection of 200 or 50 μg/site of poly-L-arginine, respectively. The scratching around the injected site with the hind-paws was counted for 30 min after the intradermal injection. Each value represents the mean ± S.E.M. from 8 to 9 animals. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the value in the vehicle-treated group.

(times/30 min) were 1 ± 0, 6 ± 5, 37 ± 15 and 77 ± 14 (mean ± S.E.M., $n = 4$) following 0, 50, 100 and 200 μg/site of poly-L-arginine, respectively. In the subsequent experiments, 200 μg/site of poly-L-arginine was used since this dose produced the scratching behavior sufficient to assess the effects of drugs.

Poly-L-aspartic acid (2, 20 and 200 μg/site, intradermally) inhibited the scratching behavior with the increase of the injected dose, and completely inhibited it at 200 μg/site. The numbers of scratching (times/30 min) were 37 ± 8 for the control, 27 ± 8 at 2 μg/site, 25 ± 5 at 20 μg/site and 5 ± 1 at 200 μg/site (mean ± S.E.M., $n = 8$), respectively. Naloxone (1 mg/kg, i.p.), an opioid receptor antagonist, suppressed the poly-L-arginine-induced scratching behavior by 75.0% (Fig. 4).

An intradermal injection of histamine (0.005–500 μg/site) or substance P (30–300 μg/site) failed to induce scratching behavior (Fig. 5A and B). Intradermal injections of the lower doses (0.005–5 μg/site) of substance P also did not elicit scratching behavior. The numbers of scratching (times/30 min) were 2 ± 0, 0 ± 0, 2 ± 2, 3 ± 3 and 3 ± 2 (mean ± S.E.M., $n = 3$) following 0, 0.005, 0.05, 0.5 and 5 μg/site of substance P, respectively. Intradermal injections of serotonin at the high doses of 50 and 500 μg/site significantly induced scratching behavior (Fig. 5C). Methysergide at 3 mg/kg (p.o.) significantly inhibited the serotonin (500 μg/site)-induced scratching behavior. The numbers of scratching (times/30 min) were 88 ± 21 for the control, 75 ± 12 at 0.1 mg/kg, 45 ± 6 at 1 mg/kg and 27 ± 4 at 3 mg/kg (mean ± S.E.M., $n = 8$), respectively. Thereby, 3 mg/kg of methysergide was used for assessing the effects on the poly-L-arginine-induced scratching behavior.

Chlorpheniramine (10 and 30 mg/kg, p.o.) and methysergide (3 mg/kg, p.o.) did not inhibit the poly-L-arginine (200 μg/site)-induced scratching behavior (Fig. 6A and B), and their combination also failed to inhibit it (Fig. 6C).

Capsaicin desensitization (52 mg/kg, s.c.) and LY303870 (10 mg/kg, i.v.) inhibited the poly-L-arginine (200 μg/site)-induced scratching behavior by 62.8% and 95.0%, respectively (Fig. 7A and B). Phosphoramidon (2.5 mg/kg, i.v.) enhanced the poly-L-arginine (50 μg/site)-induced one by about fourfold (Fig. 7C).

In mast cell-deficient (WsRC/Slc-Ws/Ws) rats (235 ± 49 times/30 min) as well as in the control (WsRC/Slc-+/+) rats (109 ± 22 times/30 min), poly-L-arginine (200 μg/site, intradermally) induced scratching behavior (Fig. 8). The number of scratching in WsRC/Slc-Ws/Ws

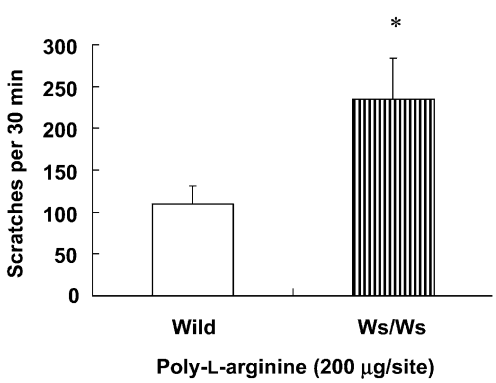

Fig. 8. Comparison of the poly-L-arginine-induced scratching behavior between the mast cell-deficient and the control rats. Poly-L-arginine (200 μg/site) was injected intradermally into the mast cell-deficient (Ws/Ws) rats and the control (Wild) ones. The scratching around the injected site with the hind-paws was counted for 30 min after the intradermal injection. Each value represents the mean ± S.E.M. from eight animals. $^{*}P < 0.05$ compared with the value in the control rats.

rats was significantly higher than that in the control rats (Fig. 8).

4. Discussion

In the present study, we observed that the cationic polypeptide poly-L-arginine induced cutaneous vascular hyperpermeability and scratching behavior, and that both responses were inhibited by poly-L-aspartic acid, a negatively charged polypeptide. These observations indicate that the poly-L-arginine-induced responses were elicited by the cationic nature of this polypeptide but not by any non-specific effect. Moreover, these responses are suggested to mimic the skin inflammation involving the endogenous polycations, like MBP.

The present study showed that the poly-L-arginine-induced vascular hyperpermeability was partially suppressed by chlorpheniramine or methysergide and was almost completely suppressed by their combination, suggesting an involvement of histamine and serotonin in the increase of vascular permeability. In the present study, moreover, poly-L-arginine only minimally increased cutaneous vascular permeability in mast cell-deficient rats as compared to the control rats. These results indicate that the poly-L-arginine-induced dye leakage is produced almost exclusively by mast cell-derived histamine and serotonin. Indeed, polycations are reported to induce histamine release from human skin mast cells (Benyon et al., 1989) and rat peritoneal mast cells (O'Donnell et al., 1983). Mast cells activated by various stimulators including basic polypeptides are also known to release serotonin as well as histamine (Uvnäs, 1974).

The present study also showed that capsaicin desensitization and the *tachykinin NK_1 receptor antagonist* LY303870 partially suppressed the poly-L-arginine-induced cutaneous vascular hyperpermeability, while the neutral endopeptidase inhibitor phosphoramidon enhanced the poly-L-arginine-induced one. These results suggest that the sensory neuropeptide substance P is involved in the vascular hyperpermeability. In fact, substance P is reported to release histamine from rat peritoneal mast cells via *tachykinin NK_1 receptors* (Ogawa et al., 1999). Collectively, poly-L-arginine is assumed to induce vascular hyperpermeability at least partly by releasing substance P from capsaicin-sensitive sensory nerves, resulting in the activation of mast cells to release histamine and serotonin. Indeed, polycations are shown to release substance P from rat dorsal root ganglia (Garland et al., 1997) and from sensory nerves (Coyle et al., 1994).

We found that poly-L-arginine induced scratching behavior in rats. Andoh et al. (1998) demonstrated that the substance P-induced scratching behavior in mice was an itch-associated reaction because the opioid antagonist naloxone suppressed the scratching behavior. Naloxone is known to inhibit the pruritus, which is the most common side effect associated with the epidural or intrathecal injection of opioid analgesics, especially morphine (Saiah et al., 1994). In addition, naloxone ameliorates itch sensation of the patients with pruritic diseases (Bernstein and Swift, 1979; Bergasa et al., 1992), and inhibits the scratching of the patients (Bergasa et al., 1992). In this study, the poly-L-arginine-induced scratching behavior was also depressed by naloxone. Thereby, it is supposed that the poly-L-arginine-induced scratching behavior is an itch-related reaction.

The present study indicated that neither chlorpheniramine nor methysergide affected the poly-L-arginine-induced scratching behavior, suggesting that the activation of histamine H_1 or *5-HT receptors* is not involved in the scratching behavior in rats. In addition, exogenous histamine (0.005–500 μg/site) failed to induce scratching behavior, and exogenous serotonin only at its high doses (50 and 500 μg/site) induced scratching behavior. Furthermore, poly-L-arginine elicited scratching behavior even in mast cell-deficient (WsRC/Slc-Ws/Ws) rats, and the frequency of scratching was even higher than that of control rats. These results indicate that histamine or serotonin, derived from mast cells, is not essential to the poly-L-arginine-induced scratching behavior in rats, though both amines are known as itch mediators in humans (Hägermark, 1974; Weisshaar et al., 1997) and in some strain of mice (Inagaki et al., 1999; Yamaguchi et al., 1999). Although it is not clear why the number of scratching in the mast cell-deficient rats was higher than that in the control ones, the deficit of the anion heparin due to the lack of mast cells may have contributed to the higher frequency of scratching.

Capsaicin desensitization and LY303870 significantly suppressed the poly-L-arginine-induced scratching behavior, while phosphoramidon enhanced the poly-L-arginine-induced one. In the in vitro (Garland et al., 1997) and in vivo (Coyle et al., 1994) experiments, polycations have been shown to induce substance P release from sensory nerves. These findings suggest that the release of substance P from *sensory nerves* is involved in the poly-L-arginine-induced scratching behavior. On the other hand, intradermal injection of substance P did not induce significant scratching behavior in rats, suggesting that this peptide is not a main peripheral mediator for the poly-L-arginine-induced scratching behavior. Substance P is known to be a neurotransmitter not only in the afferent sensory neurons in the skin but also in the spinal cord (Otsuka and Yanagisawa, 1990). In fact, the *tachykinin NK_1 receptors* are expressed in the rat spinal cord (Yashpal et al., 1990; Moussaoui et al., 1992). Moreover, substance P is reported to facilitate the activation of spinal dorsal horn neurons following noxious peripheral stimuli (Henry, 1976). Similarly, substance P is assumed to mediate at the level of spinal cord the transmission of itch sense from the periphery to the brain in the poly-L-arginine-induced scratching behavior. It is thus suggested that LY303870, acting on the

tachykinin NK_1 receptors in the spinal cord, inhibited the poly-L-arginine-induced scratching behavior. Indeed, LY303870 is a centrally active *tachykinin NK_1 antagonist* (Iyengar et al., 1997). Accordingly, we hypothesize that, in the poly-L-arginine-induced scratching, substance P acts as a spinal transmitter. This hypothesis is partly based on the desensitizing effect of capsaicin on the scratching behavior. However, since ablation of capsaicin-sensitive neurons is widely known to have consequences on a range of afferent modalities (McMahon and Koltzenburg, 1992), there remains a possibility that the other mediators than substance P are involved in the response. Further studies are required to confirm the present hypothesis of substance P as a spinal transmitter in the poly-L-arginine-induced scratching.

The present findings that poly-L-arginine induced the scratching behavior as well as the vascular hyperpermeability suggest that the polycation, possibly derived from eosinophils, is one of the mediators responsible for the pathogenesis of pruritic dermatitis. This is supported by the observation that MBP is deposited in the skin of the patients with urticaria or atopic dermatitis (Peters et al., 1983; Leiferman, 1989). The present results also suggest that histamine and serotonin from mast cells and substance P from *sensory nerves* play crucial roles in the vascular hyperpermeability and the itch of the pruritic dermatitis involving MBP. In fact, repeated topical treatment with capsaicin is reported to ameliorate pruritus in the patients with urticaria or atopic dermatitis (Cappugi et al., 1989). Thus, the presently clarified, poly-L-arginine-induced response in rats seems to be an appropriate animal model to investigate the pathophysiology of the pruritic dermatitis involving polycations and to examine the effects of drugs on this disease as well.

In conclusion, the present study demonstrated that poly-L-arginine induced scratching behavior, which may be an itch-associated response, in addition to cutaneous vascular hyperpermeability in rats. The present results suggest that both the cutaneous vascular hyperpermeability and the scratching behavior are at least partly mediated by the sensory neuropeptide substance P. Additionally, mast cell-derived histamine and serotonin are suggested to be involved in the cutaneous vascular hyperpermeability but scarcely, if any, in the scratching behavior. Polycations may thus play roles in the pathogenesis of pruritic dermatitis.

References

Andoh, T., Nagasawa, T., Satoh, M., Kuraishi, Y., 1998. Substance P induction of itch-associated response mediated by cutaneous NK_1 tachykinin receptors in mice. J. Pharmacol. Exp. Ther. 286, 1140–1145.

Benyon, R.C., Robinson, C., Church, M.K., 1989. Differential release of histamine and eicosanoids from human skin mast cells activated by IgE-dependent and non-immunological stimuli. Br. J. Pharmacol. 97, 898–904.

Bergasa, N.V., Talbot, T.L., Alling, D.W., Schmitt, J.M., Walker, E.C., Baker, B.L., Korenman, J.C., Park, Y., Hoofnagle, J.H., Jones, E.A., 1992. A controlled trial of naloxone infusions for the pruritus of chronic cholestasis. Gastroenterology 102, 544–549.

Bernstein, J.E., Swift, R., 1979. Relief of intractable pruritus with naloxone. Arch. Dermatol. 115, 1366–1367.

Cappugi, P., Lotti, T., Tsampau, D., Danesi, A., Zippi, P., Bonan, P., Panconesi, E., 1989. Capsaicin treatment of different dermatological affections with itching. Skin Pharmacol. 2, 230.

Coyle, A.J., Ackerman, S.J., Irvin, C.G., 1993. Cationic proteins induce airway hyperresponsiveness dependent on charge interactions. Am. Rev. Respir. Dis. 147, 896–900.

Coyle, A.J., Perretti, F., Manzini, S., Irvin, C.G., 1994. Cationic protein-induced sensory nerve activation: role of substance P in airway hyperresponsiveness and plasma protein extravasation. J. Clin. Invest. 94, 2301–2306.

Del Monte, M., Omini, C., Subissi, A., 1990. Ganglion-blocking agents enhance neurally mediated bronchoconstriction in the guinea-pig: possible role of sensory neuropeptides. Eur. J. Pharmacol. 180, 239–245.

Foreman, J.C., Jordan, C.C., Oehme, P., Renner, H., 1983. Structure–activity relationships for some substance P-related peptides that cause wheal and flare reactions in human skin. J. Physiol. 335, 449–465.

Garland, A., Necheles, J., White, S.R., Neeley, S.P., Leff, A.R., Carson, S.S., Alger, L.E., McAllister, K., Solway, J., 1997. Activated eosinophils elicit substance P release from cultured dorsal root ganglion neurons. Am. J. Physiol. 273, L1096–L1102.

Hägermark, Ö., 1974. Studies on experimental itch induced by kallikrein and bradykinin. Acta Dermatovenerol. (Stockholm) 54, 397–400.

Hägermark, Ö., Hökfelt, T., Pernow, B., 1978. Flare and itch induced by substance P in human skin. J. Invest. Dermatol. 71, 233–235.

Hashimoto, M., Yamamoto, Y., Takagi, H., 1997. Effects of KB-2796 on plasma extravasation following antidromic trigeminal stimulation in the rat. Res. Commun. Mol. Pathol. Pharmacol. 97, 79–94.

Henry, J.L., 1976. Effects of substance P on functionally identified units in cat spinal cord. Brain Res. 114, 439–451.

Inagaki, N., Nakamura, N., Nagao, M., Musoh, K., Kawasaki, H., Nagai, H., 1999. Participation of histamine H_1 and H_2 receptors in passive cutaneous anaphylaxis-induced scratching behavior in ICR mice. Eur. J. Pharmacol. 367, 361–371.

Iyengar, S., Hipskind, P.A., Gehlert, D.R., Schober, D., Lobb, K.L., Nixon, J.A., Helton, D.R., Kallman, M.J., Boucher, S., Couture, R., Li, D.L., Simmons, R.M.A., 1997. LY303870, a centrally active neurokinin-1 antagonist with a long duration of action. J. Pharmacol. Exp. Ther. 280, 774–785.

Katayama, S., Shionoya, H., Ohtake, S., 1978. A new method for extraction of extravasated dye in the skin and the influence of fasting stress on passive cutaneous anaphylaxis in guinea pigs and rats. Microbiol. Immunol. 22, 89–101.

Leiferman, K.M., 1989. Eosinophils in atopic dermatitis. Allergy 44 (Suppl. 9), 20–26.

Leiferman, K.M., Loegering, D.A., Gleich, G.J., 1984. Production of wheal-and-flare skin reactions by eosinophil granule proteins. J. Invest. Dermatol. 82, 414.

McMahon, S.B., Koltzenburg, M., 1992. Itching for an explanation. Trends Neurosci. 15, 497–501.

Moussaoui, S.M., Hermans, E., Mathieu, A.M., Bonici, B., Clerc, F., Guinet, F., Garret, C., Landuron, P.M., 1992. Polyclonal antibodies against the rat NK-1 receptor: characterization and localization in the spinal cord. Mol. Neurosci. Neurorep. 3, 1073–1076.

Needham, L., Hellewell, P.G., Williams, T.J., Gordon, J.L., 1988. Endothelial functional responses and increased vascular permeability induced by polycations. Lab. Invest. 59, 538–548.

O'Donnell, M.C., Ackerman, S.J., Gleich, G.J., Thomas, L.L., 1983. Activation of basophil and mast cell histamine release by eosinophil granule major basic protein. J. Exp. Med. 157, 1981–1991.

Ogawa, K., Nabe, T., Yamamura, H., Kohno, S., 1999. Nanomolar

concentrations of neuropeptides induce histamine release from peritoneal mast cells of a substrain of Wistar rats. Eur. J. Pharmacol. 374, 285–291.

Otsuka, M., Yanagisawa, M., 1990. Pain and neurotransmitters. Cell. Mol. Neurobiol. 10, 293–302.

Peters, M.S., Schroeter, A.L., Kephart, G.M., Gleich, G.J., 1983. Localization of eosinophil granule major basic protein in chronic urticaria. J. Invest. Dermatol. 81, 39–43.

Pini, L.A., Vitale, G., Ottani, A., Sandrini, M., 1997. Naloxone-reversible antinociception by paracetamol in the rat. J. Pharmacol. Exp. Ther. 280, 934–940.

Saiah, M., Borgeat, A., Wilder-Smith, O.H.G., Rifat, K., Suter, P.M., 1994. Epidural-morphine-induced pruritus: propofol versus naloxone. Anesth. Analg. 78, 1110–1113.

Stein, O., De Vries, A., Katchalski, E., 1956. The effect of polyamino acids on the blood vessels of the rat. Arch. Int. Pharmacodyn. 57, 243–253.

Uchida, D.A., Ackerman, S.J., Coyle, A.J., Larsen, G.L., Weller, P.F., Freed, J., Irvin, C.G., 1993. The effect of human eosinophil granule major basic protein on airway responsiveness in the rat in vivo. A comparison with polycations. Am. Rev. Respir. Dis. 147, 982–988.

Uvnäs, B., 1974. Histamine storage and release. Fed. Proc. 33, 2172–2176.

Wasmoen, T.L., Bell, M.P., Loegering, D.A., Gleich, G.J., Prendergast, F.G., McKean, D.J., 1988. Biochemical and amino acid sequence analysis of human eosinophil granule major basic protein. J. Biol. Chem. 263, 12559–12563.

Weisshaar, E., Ziethen, B., Gollnick, H., 1997. Can a serotonin type 3 (5-HT3) receptor antagonist reduce experimentally-induced itch. Inflammation Res. 46, 412–416.

Yamaguchi, T., Nagasawa, T., Satoh, M., Kuraishi, Y., 1999. Itch-associated response induced by intradermal serotonin through 5-HT_2 receptors in mice. Neurosci. Res. 35, 77–83.

Yashpal, K., Dam, T.V., Quirion, R., 1990. Quantitative autoradiographic distribution of multiple neurokinin binding sites in rat spinal cord. Brain Res. 506, 259–266.