Evaluation of Organic Solvent-Induced Inflammation Modulated by Neuropeptides in the Abdominal Skin of Hairless Rats

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Abstract: In this study, the severity and time course of inflammation induced by 4 organic solvents (acetone, cyclohexane, toluene and m-xylene), and the effect of neuropeptides during the inflammation were investigated in the hairless rat abdominal skin. Plasma extravasation used as a parameter of inflammation was measured by Evans blue and 125I-bovine serum albumin (BSA). Total volume of plasma extravasation induced by 4 organic solvents in 240-min exposure was as follows: toluene>m-xylene>cyclohexane>acetone=0. While hydrophobic solvents (toluene, m-xylene, cyclohexane) induced plasma extravasation, the hydrophilic solvent, acetone, did not induce plasma extravasation. It was suggested that the severity and time course of plasma extravasation depend on chemical characteristics of the organic solvents. In immunohistochemical study, substance P (SP)-immuno-reactive nerve fibers (IRNF) and calcitonin gene-related peptide (CGRP) -IRNF were intact during 240-min exposure to acetone. In contrast, cyclohexane, toluene, and m-xylene reduced the number of SP-IRNF and CGRP-IRNF in 10 min exposure and further reduced immunoreactivity. In hairless rats treated with systemic capsaicin, the above plasma extravasation was significantly reduced, along with SP-IRNF and CGRP-IRNF; however, protein gene product 9.5 (PGP 9.5) -IRNF was nearly intact. These results indicated that certain organic solvents induce instance of inflammation that vary widely in terms of their severity and time course, and that these differences are correlated with neuropeptides.

Key words: Organic solvent, Neurogenic inflammation, Plasma extravasation, Neuropeptide, Substance P, Calcitonin gene-related peptide (CGRP), Capsaicin, Hairless rat abdominal skin

Introduction

It is well known that many organic solvents induce irritant inflammation in the skin. The Academy of Dermatology identified occupational skin disease as one of the most perspective health problem in American work environments1. Of all work-related diseases and injuries, skin damage caused by plants and chemicals ranks second in terms of frequency of occurrence and economic consequences1. Cutaneous irritation accounts for 80% of all cases of chemically induced dermatitis5. Dermatitis from organic solvents almost always results from contact irritation rather than allergy9. Cutaneous irritation causes inflammatory reaction. There have been few published reports on acute inflammation induced by organic solvents. Some investigator have applied chemicals to the ears2,4) and the back5,6) of animals and measured the skin thickness for irritation. As this method is not accurate nor objective, however, it is not suitable for checking the
acute phase of inflammation. Another problem of this method is that most of organic solvents are volatile, so it is difficult to keep the organic solvents in contact with the same volume of an exposed area. On the other hand, measuring plasma extravasation using isotope-labeled albumin is a very accurate and objective method for evaluating the severity of inflammatory responses, and is commonly used in pharmacological studies on the efficiency of anti-inflammatory drugs. Veronesi et al. reported that early symptoms of chemically induced skin irritation are neurally mediated. Neurogenic inflammation is a type of inflammation caused by antidromic stimulation of the unmyelinated C-fibers or thinly myelinated Aδ sensory fibers; for example, by application of irritant chemicals. Polymodal nociceptor is sensitive to various stimuli, including noxious chemicals. When polymodal nociceptors detect noxious stimuli that are potentially or actually harmful to the tissue, nerve impulse will travel to the central nervous systems (spinal cord or brainstem) to produce a sensation and initiate autonomic homeostatic reflexes. In addition, neuropeptide, such as tachykinin (e.g. substance P, Neurokinin A), and calcitonin gene-related peptide (CGRP) can be released from their peripheral endings by axon reflex in order to control the function of adjacent effector systems; for example, blood vessels. This “antidromic vasodilation” is associated with an increase in vascular permeability leading to plasma extravasation and formation of edema. Chemical substances in contact with the skin surface and cutaneous inflammatory processes, which involve complex chemical events, evoke various sensory experiences, including pain and hyperalgesia. While organic solvents may stimulate polymodal nociceptor and release some neuropeptides, there are few reports on the specific mechanism of inflammation induced by the organic solvents that widely used in industry.

The purpose of this study is to investigate the severity and time course of inflammations induced by four organic solvents (acetone, cyclohexane, toluene, and m-xylene), with a focus on neuropeptides in the inflammations. Patrick et al. showed that differences in vehicles did not affect the time course of irritation, but organic solvents have varying degrees of epicutaneous absorption in the skin, which will have some effects on inflammation during time exposure. The concentration of the organic solvent in blood was also measured, as it may be a parameter of the absorption ratio of those solvents. Time course of plasma extravasation induced by cutaneous exposure of the organic solvents was measured in hairless rat abdominal skin by means of Evans blue and I25 I-BSA. In immunohistochemical study, immunoreactivity of neuropeptides (SP and CGRP) was examined to determine the distribution and the change of expression during the time course of organic solvents exposure. Systemic capsaicin treatments of hairless rats that disrupt the release of neuropeptide was also used to examine the change of plasma extravasation and immunoreactivity of neuropeptides (SP and CGRP) in the hairless rat abdominal skin by an immunohistochemical method. Hairless rats were used since there is no need to shave them, thus preventing damage to the stratum corneum, which is the most important barrier in the skin.

Materials and Methods

Reagents
Evans blue, capsaicin and Tween 80 (polyoxyethylene sorbitan monooleate) were obtained from Sigma Chemical (MO USA). I25 I-BSA was obtained from ICN (CA, USA). Acetone, cyclohexane, toluene, and m-xylene were obtained from Wako Pure Chemical (Osaka, Japan). The histochemical antibody for SP was obtained from Incstar (MN, USA). The histochemical antibody for CGRP was obtained from Genosys (Cambridge, UK). The histochemical antibody for PGP 9.5 was obtained from Ultra Clone Limited (Rossiter, UK). HISTOFINE SAB-PO (R) kit was obtained from Nichirei (Tokyo, Japan).

Animals and exposure to organic solvents
Hairless rats (male, 9–10 W, 220 ± 10 g wt, WBN/ILA-Ht) were obtained from Saitama Experimental Animals supply Co. Ltd. (Saitama, Japan). The hairless rats were anesthetized with pentobarbital (30 mg/kg) intraperitoneally. Chambers (i.d.2 cm) were glued to the abdominal skin using cyanoacrylate glue (Konishi, Japan). Polyethylene catheters were cannulated into the carotid artery, the jugular vein, and the trachea. After each organic solvent (1 ml) was injected into a chamber, the chamber was sealed immediately.

Measurement of organic solvents concentration
The organic solvent concentration in blood was measured...
by a slightly modified version of the method developed by Jakobson et al.\textsuperscript{12, 13}) Anesthesia and exposure to organic solvents was as described above. One chamber was glued to the abdominal skin of the hairless rats. Six to eight hairless rats were placed each organic solvents exposure group. Blood samples (0.3 ml) were withdrawn into syringes via catheter from the carotid artery at 5, 10, 20, 30, 60, 120, 240-min after exposure. The blood loss was compensated for by injecting Haemaccell (Behring Werke, Germany) via the catheter into the jugular vein. The blood samples were injected directly into glass vials, which were sealed with a rubber membrane and aluminum cap. The analysis was performed using a head-space gas chromatograph (GC-17A Shimadzu, Japan). A 3 m glass column (i.d. 2.6 mm) packed with PEG-20M (GL Science, Japan) was used for GC analysis. The analytical GC conditions were as follows: temperature of the column was 80°C, flow rate of carrier gas (N\textsubscript{2}) was 30 ml/min, and temperature of detector (FID) was 225°C. The head-space bottles for GC were incubated for 30 min at 60°C for equilibration.

**Vascular permeability**

Local plasma extravasation in hairless rat abdominal skin was measured using the modified version of the methods\textsuperscript{7-9} based on the accumulation of intravenously injected Evans blue dye and \textsuperscript{125}I-BSA. Two percent Evans blue in saline (2 ml/kg) was injected into the jugular vein 10 min before exposure of organic solvents. Saline was used as the control. Anesthesia and exposure to organic solvents was as described above. Five chambers were glued on the abdominal skin of each hairless rat, and saline, acetone, cyclohexane, toluene and m-xylene were injected into each chamber. There were 2 animals in each exposure time group. After organic solvent exposure (10, 30, 60, 240 min), the animals were sacrificed using pentobarbital injections. After the chambers were removed, the abdominal skin was removed and examined for blue staining.

Leakage of \textsuperscript{125}I-BSA was used to quantify changes in vascular permeability. Anesthesia and exposure to organic solvents was as described above. Two chambers were glued on the abdominal skin of each hairless rat, then saline and organic solvent were injected into each chamber. \textsuperscript{125}I-BSA (2 µCi/kg) with 2 ml/kg of Evans blue dye was injected via catheter 10 min before organic solvents were injected into chambers. The length of organic solvent experiments were based on the results of Evans blue studies (30, 60, 120, 240 min). There were 6–8 animals in each time and organic solvents exposure group. A 3 ml of blood sample was taken from the cardiac artery into heparin (10 U/ml) just before the end of exposure. One ml of plasma was prepared from this blood by centrifugation (8,000 g for 5 min). After the animals sacrificed using pentobarbital injection, the skin sites were punched out with steel punch (i.d. 15 mm). Plasma and skin samples were counted by Gamma counter (Cobra Quantum 5002. Packard. CT, USA). In order to exclude cyanoacrylate glue and physiological effect, the amount of plasma extravasation in each sample was calculated from the following formula:

\[
\text{Skin plasma volume} = \frac{(\text{skin sample count/5 min} - \text{control (saline) skin sample count/5 min})}{1 \mu l \text{ of plasma count/5 min}}
\]

**Capsaicin pretreatment**

The hairless rats were anaesthetized with ethyl ether and were injected subcutaneously with 50 mg/kg of capsaicin solution (dissolved in 10% ethanol, 10% Tween 80, 80% physiological saline) in the dorsal sites. The hairless rats were treated on the 4 following conservative days with increasing dosages (100, 200, 200, and 400 mg/kg) of capsaicin, or with the same amount of vehicle as Harti et al.\textsuperscript{18} The hairless rats were tested for insensitivity to capsaicin using the wiping test\textsuperscript{19} at 3 days after the last injection and then were used in neurophysiological and immunochemical experiments. In brief, 0.01% of capsaicin solution was instilled into the right eyes of the hairless rats. The number of wipings in the first 2 min after this treatment served as an index of desensitization.

**Immunohistochemical study**

Anesthesia and exposure to organic solvents was as described above. Two animals were used each time and organic solvents exposure group (Total 48 rats). After 10, 30, 60, 240 min of exposure, animals were sacrificed using pentobarbital injections. The hairless rat abdominal skin was punched out with a standard steel punch (i.d.8 mm), and the tissues were fixed overnight in 2% paraformaldehyde at 4°C, after which they were immersed overnight in phosphate-buffered saline (PBS) containing 20% sucrose. After embedding in an OTC compound, they were frozen immediately using liquid nitrogen and then kept at −70°C. The issues were then cut on a cryostat at 20 µm. The immunohistochemical reaction was carried out using the streptavidin biotin (SAB) technique. Briefly, after rinsing in PBS, the tissues were incubated in anti-SP, anti-CGRP, and anti-protein gene product 9.5 (PGP 9.5) polyclonal.
antibodies overnight at 37°C. The dilution of all primary antibodies was 1:3,000. After incubation in primary antiserum, the tissue sections were sequentially incubated in biotinylated goat anti-rabbit IgG and streptavidin-biotin-peroxidase complex. The reaction product was visualized by using hydrogen peroxide and diaminobenzidine as a chromogen. Weak to strong stained nerve fibers were considered positive, the number of immunoreactive fibers of the main nerve branches innervating the abdominal skin of hairless rats were counted.

Statistics
Statistical analysis was performed using one-way Factorial ANOVA and Fisher's PLSD was used to compare 4 organic solvents.

Results

Organic solvents concentration in blood
Toluene and m-xylene concentration in blood increased with time and reached plateau after 120 min (Fig. 1). Cyclohexane concentration increased up to 1 h and then decreased until the end of exposure. The blood concentration of acetone was increased linearly to the end of exposure.

Time course of the plasma extravasation of 4 organic solvents
After 10 min exposure to cyclohexane, toluene and m-xylene, speckles of blue dye began to appear in the skin area (Fig. 2a). The speckles then rapidly enlarged and coarsened so that the sites quickly became evenly colored during exposure of those 3 organic solvents (Fig. 2b–d). Toluene induced the darkest color among 4 organic solvents. While acetone- and saline-exposed skin was not dyed during 240-min exposure (Fig. 2a–d).

Figure 3 shows the time course of plasma extravasation. Toluene was the most potentate inducer of plasma extravasation. Plasma extravasation by toluene increased rapidly in the first 30 min and then slowed during the remainder of 240 min. m-Xylene induced less plasma extravasation than toluene, but responded very similarly in terms of time course to toluene. Plasma extravasation by cyclohexane increased up to 120 min and then decreased slightly. The effect of acetone was marginal compared to the saline-treated control site. The results of plasma extravasation induction in 240-min exposure was as follows: toluene>m-xylene>cyclohexane>acetone=0. (p<0.01). (240 min; acetone: n=8, mean=0.31, SD=0.95, cyclohexane: n=8, mean=76.11, SD=24.22, toluene: n=8, mean=239.61, SD=23.59, m-xylene: n=8, mean=179.60, SD=6.22.)

Behavioral controls
The number of wiping reactions was greatly reduced after systemic capsaicin treatment (Before: mean=24.6, SD=8.0 After: mean=1.6, SD=1.8, n=41, P<0.0001).

Effect of systemic capsaicin treatment
Based on the results of plasma extravasation of 4 organic solvents and the control (saline), toluene and cyclohexane was chosen for this study. This was due to the fact that toluene and m-xylene belong to the same aromatic hydrocarbons group and they displayed similar patterns in time course of plasma extravasation. Acetone and saline did not induce plasma extravasation during 240-min exposure.

In systemic capsaicin treatment of hairless rats, plasma extravasation induced by cyclohexane was reduced by 34.4% and 74.4% compared to non-capsaicin-treated hairless rats.

![Fig. 1. Time course of epicutaneous absorption of 4 organic solvents](image-url)
Each value represents the mean and SD. Acetone used right side bar. Cyclohexane, toluene and m-xylene used left side bar. n=6 (acetone, cyclohexane, m-xylene), n=8 (toluene).
during 60-min and 240-min exposure to cyclohexane, respectively (Fig. 4). On the other hand, plasma extravasation induced by toluene was significantly reduced by 42.2% and 46.4% compared to non-capsaicin-treated hairless rats during 60-min and 240-min exposure to toluene, respectively (Fig. 5).

**Immunohistochemical study**

During immunohistochemical study, SP-IRNF, CGRP-IRNF and PGP 9.5-IRNF were present in free nerve endings in the dermal papillae and in the epidermis acetone-exposed hairless rat abdominal skin, which displayed no remarkable difference during 240-min exposure (Fig. 6a, b). In control

![Fig. 2. Time course of plasma extravasation of 4 organic solvents in hairless rat abdominal skin](image)

**Fig. 2.** Time course of plasma extravasation of 4 organic solvents in hairless rat abdominal skin

a; after 10 min exposure, b; after 30 min exposure, c; after 60 min exposure, d; 240 min exposure.

![Fig. 3. Time course of plasma extravasation of 4 organic solvents by 125I-BSA](image)

**Fig. 3.** Time course of plasma extravasation of 4 organic solvents by 125I-BSA

Each value represents the mean and SD. 30min; acetone: n=2, mean=0.31, SD=0.91, cyclohexane: n=8, mean=38.39, SD=7.3, toluene: n=8, mean=94.48, SD=15.60, m-xylene: n=6, mean=46.78, SD=16.12. 60min; acetone: n=2, mean=0.65, SD=0.78, cyclohexane: n=8, mean=38.51, SD=7.3, toluene: n=8, mean=122.65, SD=15.06, m-xylene: n=8, mean=65.59, SD=31.85. 120min; acetone: n=2, mean=0.73, SD=0.64, cyclohexane: n=8, mean=103.69, SD=27.35, toluene: n=8, mean=122.78, SD=25.35, m-xylene: n=8, mean=179.60, SD=6.22.
ORGANIC SOLVENT-INDUCED INFLAMMATION MODULATED BY NEUROPEPTIDES

(saline treated) skin, SP-IRNF, CGRP-IRNF and PGP 9.5-IRNF were also present in free nerve endings in dermal papillae and in the epidermis in acetone exposed hairless rat abdominal skin that was no remarkable difference during 240-min exposure (Table 1–3). SP-IRNF and CGRP-IRNF had a very thin and varicose appearance and exhibited a branching pattern in certain places (Fig. 6a, b). Most of these terminals were observed beneath the basal layer of the epidermis and in the dermal papillae, and some penetrated the epidermal layer, even reaching the limit of the stratum corneum (×400). In b, CGRP-IRNF were observed beneath the basal layer of the epidermis and in the dermal papillae, and some penetrated the epidermal layer and some CGRP-IRNF were present around appendage in the dermis (×200). Scale 25 μm.

In systemic capsaicin treatment of hairless rat abdominal skin, while SP-IRNF were still present in deep dermis and subcutaneous tissue (Table 1). The number of SP-IRNF in deep dermis and subcutaneous tissue were gradually decreased, and became less intense and less homogeneous during time course of cyclohexane, toluene and m-xylene exposure (Fig. 7a). The number of CGRP-IRNF were decreased more lenient during exposure than that of SP-IRNF (Table 2, Fig. 7b). PGP 9.5-IRNF remained intact compared to SP-IRNF and CGRP-IRNF (Fig. 7c). The number of PGP 9.5-IRNF in epidermis and dermal papillae was reduced 240-min after cyclohexane, toluene and m-xylene exposure, but was not eliminated in comparison with SP-IRNF, CGRP-IRNF (Table 3).

In systemic capsaicin treatment of hairless rat abdominal skin, SP-IRNF, CGRP-IRNF and PGP 9.5-IRNF were also present in free nerve endings in dermal papillae and in the epidermis in acetone exposed hairless rat abdominal skin that was no remarkable difference during 240-min exposure (Table 1–3). SP-IRNF and CGRP-IRNF had a very thin and varicose appearance and exhibited a branching pattern in certain places (Fig. 6a, b). Most of these terminals were observed beneath the basal layer of the epidermis and in the dermal papillae, and some penetrated the epidermal layer, even reaching the limit of the stratum corneum (×400). In b, CGRP-IRNF were observed beneath the basal layer of the epidermis and in the dermal papillae, and some penetrated the epidermal layer and some CGRP-IRNF were present around appendage in the dermis (×200). Scale 25 μm.

Fig. 4. Effect of systemic capsaicin treatment in cyclohexane exposure
Each value represents the mean and SD. 60min; capsaicin treatment: n=8, mean=52.55, SD=11.06, vehicle: n=6, mean=124.49, SD=12.06, 240min; capsaicin treatment: n=8, mean=93.75, SD=19.49, vehicle: n=6, mean=202.24, SD=51.09.

Fig. 5. Effect of systemic capsaicin treatment in toluene exposure
Each value represents the mean and SD. 60min; capsaicin treatment: n=8, mean=14.47, SD=7.78, vehicle: n=6, mean=41.98, SD=7.93, 240min; capsaicin treatment: n=8, mean=61.40, SD=27.82, vehicle: n=6, mean=83.56, SD=18.76.

Fig. 6. Immunoreactivity of SP and CGRP in the hairless rat abdominal skin during 240-min exposure to acetone
In a, SP-IRNF were observed beneath the basal layer of the epidermis and in the dermal papillae, and some penetrated the epidermal layer, even reached the limit of the stratum corneum (×400). In b, CGRP-IRNF were observed beneath the basal layer of the epidermis and in the dermal papillae, and some penetrated the epidermal layer and some CGRP-IRNF were present around appendage in the dermis (×200). Scale 25 μm.
The number of SP-IRNF and CGRP-IRNF significantly decreased (Fig. 8a), while PGP 9.5-IRNF was intact in the epidermis, dermal papillae (Fig. 8b), deep dermis, and subcutaneous tissue. In the abdominal skin of vehicle (10% ethanol, 10% Tween 80, 80% physiological saline) treated hairless rats, SP, CGRP, and PGP 9.5 immunoreactivity displayed no remarkable changes compared to the abdominal skin of non-treated hairless rats.

**Discussion**

Neurogenic inflammation is considered to be a potent endogenous defense mechanism during the early phase of tissue damage, as the enhanced blood flow and the plasma extravasation dilute and remove the affecting substances. Measurement of plasma extravasation volume was used for evaluating the severity of inflammation. Organic solvents...

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**Table 1. Distribution and time course of SP-IRNF in hairless rat abdominal skin**

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<td>Control (Saline)</td>
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++: Easy to find positive nerve fibers in high magnification fields (× 400) and there is no remarkable change compared to control. +: Easy to find positive nerve fibers in high magnification fields (× 400) but reduced the number of positive nerve fibers (less than 75%) compared to control. ±: Difficult to find positive nerve fibers in high magnification fields (× 400) but positive nerve fibers can be found in each specimen. —: No positive nerve fibers can be found in each specimen.

**Table 2. Distribution and time course of CGRP-IRNF in hairless rat abdominal skin**

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inducing the change of vascular permeability caused plasma extravasation, which is an important feature of inflammation\textsuperscript{11}. Plasma extravasation induced by 4 organic solvents was varied in the time course of the response (Fig. 3). Hydrophobic solvents, such as cyclohexane, toluene, and m-xylene induced plasma extravasation. Toluene, which is one of the most commonly used organic solvents, was the most potent inducer of plasma extravasation among these 4 solvents. m-Xylene induced less plasma extravasation than toluene, but more than cyclohexane. The time course of plasma extravasation induced by m-xylene was very similar in pattern to that by toluene. The similarity of chemical structure found in toluene and m-xylene may be reason for the similar inflammation patterns. Plasma extravasation induced by cyclohexane, which was less than that of aromatic compounds, (toluene and m-xylene), increased up until 120 min and then gradually decreased while aromatic compounds kept increasing plasma extravasation for the entire 240 min. Toluene and cyclohexane caused rapid histological changes such as perinuclear edema, karyopycnosis, spongiosis, intra-epidermal vesicles and marked epidermal-dermal separation\textsuperscript{21}. Boman \textit{et al.} indicated that acute injuries reduced epicutaneous absorption in hydrophobic solvents including toluene, but increased epicutaneous absorption in hydrophilic solvents, and also concluded that intercellular edema (spongiosis) may act as a secondary partitioning barrier for hydrophobic solvents\textsuperscript{13}. In addition, inflammatory response, including plasma extravasation, is caused mainly by postcapillary venules, which are located in upper plexus of the skin near the dermal papillae\textsuperscript{22}. Fluids from postcapillary venules pools gradually (edema) in the tissues in the time course of exposure. Both spongiosis in the epidermis and edema in the upper dermis were found in the cyclohexane- and toluene-exposed skin (data was not shown). Spongiosis and edema may inhibit epicutaneous absorption of cyclohexane to a greater extent than toluene since cyclohexane (Octanol/Water partition coefficient, log $P=3.44$) is less soluble in water than toluene (Octanol/Water partition coefficient, log $P=2.69$)\textsuperscript{36} (Fig. 1). Inhibition of epicutaneous absorption may result in less stimulation of polymodal nociceptor and fewer products of chemical agents, e.g., bradykinin, which is a effective excitant of C fiber polymodal nocicepters. In systemic capsaicin treatments of hairless rat, plasma extravasation by cyclohexane and toluene was reduced more than non-capsaicin-treated hairless rats, although plasma extravasation during 240-min exposure to cyclohexane was reduced less than others (Figs. 4 and 5). Differences in the Octanol/Water partition coefficient may also result in different inhibitions during 60-min and 240-min exposure to cyclohexane in systemic capsaicin treatments of hairless rats compared to toluene. These results reconfirmed that systemic capsaicin treatment of adult animals leads to changes in plasma extravasation and neuropeptide depletion in various organs and tissue\textsuperscript{14,16}. Acetone did not cause plasma extravasation and the

### Table 3. Distribution and time course of PGP9.5-IRNF in hairless rat abdominal skin

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++: Easy to find positive nerve fibers in high magnification fields ($\times 400$) and there is no remarkable change compared to control. +: Easy to find positive nerve fibers in high magnification fields ($\times 400$) but reduced the number of positive nerve fibers compared to control. ±: Difficult to find positive nerve fibers in high magnification fields ($\times 400$) but positive nerve fibers can be found in each specimen. −: No positive nerve fibers can be found in each specimen.
immunohistochemical study showed acetone did not affect the immunoreactivity of SP and CGRP in hairless rat abdominal skin. This result suggests that acetone does not affect the release of neuropeptides and plasma extravasation. Acetone is a hydrophilic solvent and is not typically described as causing significant histological change. The results of plasma extravasation induced by 4 organic solvents also indicated hydrophobic solvents (cyclohexane, toluene, m-xylene) induces plasma extravasation contrary to hydrophilic solvent (acetone).

On the other hand, the blood concentration of acetone increased linearly and was the highest during exposure to the other organic solvents (Fig. 1). Boman et al. reported that the blood concentration of butanol (hydrophilic solvents) was the highest in other hydrophobic solvents (toluene, 1,1,1-trichloroethane). In this study, epicutaneous absorption did not seem to affect inflammation. On the contrary, inflammation seemed to affect its epicutaneous absorption in hydrophobic solvents, but not in hydrophilic solvents.

During immunohistochemical study, SP-IRNF and CGRP-IRNF decreased in inverse proportion to the amount of plasma extravasation induced by cyclohexane, toluene, and m-
It was suggested that neuropeptides were released by certain organic solvents and neuropeptides were depleted in the time course of exposure to certain organic solvents. This result supports previous studies that stated neuropeptides were released from nerve endings, and that those contents in nerve endings were decreased during the neurogenic inflammation of rat skin. PGP 9.5-IRNF were intact until 60-min of exposure but sparse in 240-min exposure of cyclohexane, toluene, and m-xylene. PGP 9.5, which is a major component of the neuronal cytoplasm, was used to identify general innervation patterns. This report indicated that three organic solvents (cyclohexane, toluene and m-xylene) stimulate only nociceptors at first, and then distract nerve fibers at the end of the exposure period. It is generally accepted that, in microcirculation, SP increases microvascular leakage via stimulation of the NK1 receptor, while CGRP is an extremely potent vasodilator. CGRP coexists with SP in many primary afferent neurons and modulate the response to SP. It appears that there is a time lag between the depletion of SP and CGRP, which is due to the fact that the total amount of CGRP is larger than that of SP. As previous studies showed that CGRP localize in most, and possibly all, primary afferent neurons containing SP and also in the separate population that does not contain SP. CGRP forms the largest population of peptide-containing axons in the epidermis, dermis and around sweat glands in the skin in several species. Systemic capsaicin treatment completely deplete SP and CGRP from in cornea and only complete in the outer longitudinal muscle layer of the ureter. The depletion of substance P from sensory pathway by 125 mg/kg capsaicin in adult Sprague-Dawley rats is less than that caused by 50 mg/kg capsaicin treatment in new born rats. On the other hand, depletion of SP and CGRP is the same when capsaicin is given to newborn or adult Wister rats. In this study, only a few SP-IRNF, and CGRP-IRNF were remained in the epidermis to upper dermis. Depletion of neuropeptides seems to depend on species, strain, and organs. Previous studies showed that there is a significant reduction in the proportion of C-fiber polymodal nociceptors in the saphenous nerve following capsaicin treatment of adult rats. In addition to neuropeptides depletion, the reduction of polymodal receptors by systemic capsaicin treatments also affected the reduction of the amount of plasma extravasation by cyclohexane and toluene.

In the current study, there was no significant change in the number of PGP 9.5-IRNF by systemic capsaicin treatment, while the number of SP-IRNF and CGRP-IRNF were significantly decreased. These results support previous studies that showed that systemic capsaicin treatment of adult animals causes neuropeptide depletion in various organs and tissues. Those peripheral reactions exerted by neuropeptides may be interpreted in the context of neurogenic inflammation as protective responses.

It was concluded that certain organic solvents (cyclohexane, toluene, and m-xylene) induced the plasma extravasation that is a major feature of inflammation. The time course of changes in the immunoreactivity of neuropeptides, SP, and CGRP was also investigated. It was
suggested that these organic solvents induced a wide range of severity and time course of plasma extravasation based on their chemical structure (aromatic hydrocarbons, cycloalkanes, ketones) and were modulated by neuropeptides. Neuropeptides promote rapid peripheral reactions, including the increase of plasma extravasation, in order to prevent irritating organic solvents.

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References

ORGANIC SOLVENT-INDUCED INFLAMMATION MODULATED BY NEUROPEPTIDES


