Pharmacological Studies on the Novel Antiallergic Drug HQL-79: II. Elucidation of Mechanisms for Antiallergic and Antiasthmatic Effects

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ABSTRACT—The effects of 4-benzhydryloxy-1-3(1H-tetrazol-5-yl)-propyl) piperidine (HQL-79), a newly developed antiallergic drug, on various chemical mediators and on chemical mediator release were investigated. Orally administered HQL-79 strongly inhibited the histamine-induced skin reaction in rats, and histamine- and 5-hydroxytryptamine (5-HT)-induced bronchoconstriction in guinea pigs. HQL-79 inhibited antigen-induced release of leukotriene (LT) B₄, LTC₄, histamine and prostaglandin (PG) D₂ from the chopped lung tissues of actively sensitized guinea pigs. On the other hand, release of PGE₂, one of the bronchoprotective prostanooids, was significantly enhanced by HQL-79. In an in vivo experiment, chronic administration of HQL-79 clearly reduced PGD₂ contents and enhanced PGE₂ contents in the lungs of repeatedly antigen-exposed guinea pigs. In biochemical studies, HQL-79 inhibited mouse spleen PGD synthase in a concentration-dependent manner. None of the antiallergics such as epinastine, terfenadine, oxatomide and cetirizine inhibited the PGD synthase. HQL-79 did not affect PGE synthase in sheep vesicular gland microsomes. These results suggest that antiallergic and antiasthmatic effects of HQL-79 could be ascribed to antihistaminic- and anti-5-HT effects, chemical mediator release inhibition, PGE₂-release enhancement and PGD synthase inhibition. It is considered, in particular, that the differential modulation of PGD₂ and PGE₂ production is a conspicuous pharmacological feature of HQL-79.

Keywords: Antihistamine, Prostaglandin D₂, Prostaglandin E₂, Prostaglandin D synthase, HQL-79

Antigen-induced hypersensitivity reactions are mediated by various chemical mediators released from antigen-stimulated mast cells or basophils (1). In bronchial asthma, it has been suggested that some chemical mediators, including histamine (2, 3), leukotrienes (LT)s (4), platelet activating factor (PAF) (5), thromboxane A₂ (6) and prostaglandin (PG) D₂ (7) have significant roles in airway inflammatory responses such as airway eosinophilia, late asthmatic response and airway hyperresponsiveness as well as in immediate hypersensitivity reactions such as bronchial contraction and airway plasma extravasation.

4-Benzhydryloxy-1-3(1H-tetrazol-5-yl)-propyl) piperidine (HQL-79) is a newly synthesized antiallergic and antiasthmatic drug, which is effective in inhibiting immediate hypersensitivity reactions and airway inflammatory responses in various experimental models (8). Orally administered HQL-79 markedly inhibited passive cutaneous anaphylaxis in rats, antigen-induced bronchoconstriction and nasal vascular permeability in guinea pigs, as effectively as epinastine and ketotifen, compounds that have been established as potent antiallergics with histamine-H₁ blocking activities (9, 10). In addition to the inhibitory actions on the immediate hypersensitivity reactions, HQL-79 was effective in inhibiting asthmatic inflammatory responses such as airway eosinophilia and late-phase increase in the respiratory resistance in guinea pigs (8). These observations suggest that HQL-79 affects chemical mediators and/or their release from mast cells.

In the present study, the effects of HQL-79 on various chemical mediators and on chemical mediator release were studied both in vitro and in vivo to clarify the mechanisms for its antiallergic and antiasthmatic actions.
MATERIALS AND METHODS

Animals
Male Wistar rats, male Hartley guinea pigs and male ICR mice were purchased from Japan SLC, Inc. (Hamamatsu).

Drugs
HQL-79 and cetirizine dihydrochloride (cetirizine) were synthesized and epinastine hydrochloride (epinastine), oxatomide and terfenadine were extracted from Alesion® (Boehringer Ingelheim Japan, Kawanishi), Celtect® (Kyowa Hakko Kogyo, Tokyo) and Triludane® (Shionogi, Osaka), respectively, at High Quality-Life Research Laboratories, Sumitomo Metal Industries, Ltd. (Souraku-gun, Kyoto). Ketotifen fumarate (ketotifen) was purchased from Sigma (St. Louis, MO, USA). These drugs were suspended or dissolved in 0.5% methylcellulose for oral administration. For in vitro studies, HQL-79 was dissolved in 0.1 M tartaric acid, epinastine and ketotifen were dissolved in distilled water, cetirizine and terfenadine were dissolved in dimethylsulfoxide, and oxatomide was dissolved in 0.1 N HCl.

Contraction of isolated guinea pig ileum
Male guinea pigs (6-week-old, 320–390 g) were sacrificed by exsanguination, and the ileum was removed. The ileal segments (approximately 1.5 cm in length) were mounted in an organ bath containing 10 ml of Tyrode's solution (136.9 mM NaCl, 11.9 mM NaHCO₃, 5.6 mM glucose, 2.7 mM KCl, 1.8 mM CaCl₂, 1.1 mM MgCl₂, 0.4 mM NaH₂PO₄) aerated with a mixture of 95% O₂ and 5% CO₂ at 30°C. Changes in the tone of the preparation with 0.5 g initial resting tension were measured isotonically using an isotonic transducer (model T7-8; Orientec Japan, Tokyo) connected to an automated Magnus operation system (model IM-400; JT Tohsui, Tokyo). Test drugs were added to the organ bath 10 min before the addition of 3 × 10⁻⁵ M histamine (Wako Pure Chemicals, Osaka). The ileal contraction induced by 5-hydroxytryptamine (5-HT, Wako) was carried out at a concentration of 1 × 10⁻⁵ M. The inhibitory effects of test drugs were expressed as the percent reduction in ileal contraction compared with that measured when the ileum was treated with the vehicles.

Contraction of isolated guinea pig trachea
Male guinea pigs were sacrificed by exsanguination, and the trachea was removed. Zigzag strips of the trachea were prepared, and they were mounted in an organ bath containing 10 ml of Tyrode's solution aerated with a mixture of 95% O₂ and 5% CO₂ at 37°C. The preparations were initially loaded with 1 g tension and allowed to equilibrate before the start of the experiments. Changes in tone of the preparation were measured as described above. The preparations were contracted by 1 × 10⁻⁵ M histamine. Test drugs were applied cumulatively when the contraction response reached the plateau state. When the relaxant effect of the highest concentrations of the test drugs had reached a plateau, 3 × 10⁻⁵ M papaverine hydrochloride (Wako) was added to determine zero tissue tone. All relaxant responses by the test drugs were measured in terms of the maximal response to papaverine. The tracheal contraction induced by 5-HT was carried out at a concentration of 1 × 10⁻⁶ M. The inhibitory effects of test drugs were expressed as the percent reduction in tracheal relaxant response compared with that measured when the trachea was treated with the vehicles.

Histamine-induced cutaneous reaction in rats
Male Wistar rats (8-week-old, 175–190 g) were anesthetized by i.p. injection of 1.2 g/kg urethane (Aldrich Chemicals, Milwaukee, WI, USA) and then injected intra-dermally into the shaved back with 0.1 ml of histamine (500 µg/ml), followed by immediate i.v. injection of 1 ml of 0.5% Evans blue (Sigma) saline solution. The animals were sacrificed 30 min after the histamine-injection, and the reaction sites were removed. Evans blue was extracted and determined according to Katayama et al. (11). Test drugs were given orally 1 hr before histamine injection. The inhibitory effects of test drugs were expressed as the percent reduction in dye leakage compared with that determined in vehicle-treated animals.

Spasmogen-induced bronchoconstriction in guinea pigs
Male guinea pigs (5-week-old, 300–330 g) were anesthetized by i.p. injection of 1.5 g/kg urethane, and the trachea was cannulated. The method of measurement of bronchoconstriction was a modification of the Konzett and Rössler technique (12). The animals were artificially ventilated by a rodent respirator (Ugo Basile, Comerio, Italy) with a constant volume of 3 ml at 60 strokes/min. Changes in insufflation pressure at a constant airflow were measured by a pressure transducer (TP-400T; Nihon Kohden, Tokyo) connected to the side-arm of the tracheal cannula. Spontaneous respiratory movement was stopped by i.v. administration of 5 mg/kg gallamine triethiodide (Sigma). Bronchoconstrictions were induced by intravenous injection of the following spasmogens: 10 µg/kg histamine, 30 µg/kg 5-HT, 40 µg/kg acetylcholine (ACh, Wako), 1 µg/kg LTD₄ (Wako), 3 nmol/kg bradykinin (BK; Peptide Institute, Osaka), 3 nmol/kg neurokinin A (NKA, Peptide Institute), 10 nmol/kg substance P (SP, Peptide Institute), 150 ng/kg PAF (Wako) and 300 nmol/kg PGD₂ (Wako). In histamine- and 5-HT-induced bronchoconstrictions, test drugs were given...
orally 2 hr before spasmod injection. In bronchoconstrictions induced by other spasmodogens, test drugs were
given intravenously 15 min before spasmodogen injection. The inhibitory effects of test drugs were expressed as the
percent reduction in insufflation pressure compared with that determined in vehicle-treated animals.

**Antigen-induced release of chemical mediators from guinea pig lung tissues**

Ovalbumin (OA, Sigma) in saline solution (2 mg/ml) and Freund's complete adjuvant (Difco Laboratories,
Detroit, MI, USA) were thoroughly mixed at the ratio of 1:1 by volume, and the resultant emulsion was used for
sensitization. Male guinea pigs (5-week-old) were actively
sensitized by intradermal injection of 1 ml of the emulsion
containing 1 mg OA on their shaved backs. Three
weeks later, the animals were exsanguinated, and the
lungs were perfused with 20 ml of Ca\(^{2+}\)-free Tyrode's
solution and then removed. The lungs were chopped into
fragments (0.3 × 0.3 × 0.3 mm) with a MacIlwain tissue
chirpper (Mickle Lab. Eng. Co., Gomshall, UK). The
chopped lung tissues (200 mg wet weight) were placed in
a polypropylene tube with 2 ml of ice-cold Ca\(^{2+}\)-free
Tyrode's solution and kept on ice. The reaction tubes
containing the lung tissues were supplemented with
CaCl\(_2\) (1.8 mM), and incubated for 5 min at 37°C. After
that, the lung tissues were incubated with test drugs or
the vehicles for a further 60 min and then challenged with
OA (2 mg/ml). Fifteen minutes later, the reaction was
stopped by filtration of the medium through nylon mesh
(100 μm). LTB\(_4\), LTC\(_4\), PGD\(_2\) and PGE\(_2\) in the medium
were determined by EIA (Cayman enzyme immunoassay
kits; Cayman Chemical Company, Ann Arbor, MI,
USA). Histamine was determined fluorometrically (13).
The effects of the test drugs were expressed as the percent
inhibition or enhancement calculated as follows:

\[
\text{% inhibition} = \left(1 - \frac{(A - C)}{(B - C)}\right) \times 100 \\
\text{% enhancement} = \left(\frac{(A - C)}{(B - C)} - 1\right) \times 100
\]

where A = chemical mediator release from drug-treated
and antigen-stimulated lung tissues, B = chemical mediat-
ror release from vehicle-treated and antigen-stimulated
lung tissues, C = chemical mediator release from vehicle-
treated and unstimulated lung tissues.

**Determination of LTB\(_4\), LTC\(_4\), PGD\(_2\) and PGE\(_2\) in the
lungs of repeatedly antigen-exposed guinea pigs**

Male guinea pigs (5-week-old) were exposed to aero-
solized 1% OA (in saline) for 10 min and injected
intraperitoneally with a mixture of 10 μg OA and 20 mg
Al(OH)\(_3\) on day 1. Thereafter, the animals were exposed to the aerosolized antigen for 10 min on 3 occasions
separated by 7 days (on days 8, 15 and 22), followed
pretreatment with i.p. injection of 10 mg/kg mepyramine
maleate (mepyramine; Research Biochemicals Inter-
national, Natick, MA, USA) 30 min before antigen ex-
posure. HQL-79 was given orally 13 times from days 8 to
22: 1 hr before and 6 hr after the antigen exposure on
days 8 and 15; 1 hr before the final antigen exposure on
day 22; and once a day on days 9, 10, 13, 14, 16, 17, 20
and 21.

On day 22, 30 min after the final antigen exposure, the
animals were exsanguinated and then the lungs were per-
fused with 20 ml phosphate-buffered saline (PBS) and
removed. The excised lungs were washed with PBS and
blotted with a paper towel, and the wet weights were
measured. The lungs were cut into small pieces and
homogenized in 10 ml of ice-cold methanol using a
Polytron® homogenizer and then the homogenates were
centrifuged at 2000 × g for 15 min at 4°C. LTB\(_4\), LTC\(_4\),
PGD\(_2\) and PGE\(_2\) in the supernatant were partially purified
using a Sep-Pak C-18 reverse phase cartridge column
(Waters Japan, Tokyo) according to the methods
described in the instruction manuals of the EIA kits
before determination.

**Assays of PGD synthase and PGE synthase**

Mouse spleen PGD synthase (PGH-PGD isomerase)
was assayed by a modification of the method reported
by Christ-Hazelhof and Nugteren (14). Male mice (7 to
10-week-old, 35–44 g) were sacrificed by cervical dis-
location, and the spleens were removed. The spleens
were homogenized in 5 mM 2-mercaptoethanol (3 ml/g
spleen), and the homogenate was centrifuged at 150,000 × g
for 30 min at 4°C. The resulting supernatant was
assayed for PGD synthase. The reaction mixture
containing 200 mM Tris-HCl (pH 8.0), 0.5 mM reduced
glutathione, 0.1 mM EDTA and 30 μl of spleen super-
natant (approx. 70 μg protein) in a total volume of 180 μl
was preincubated with test drugs or the vehicles at 30°C
for 10 min. The reaction was started by the addition of 5
μM (final concentration) PGH\(_2\) (Wako), and 2 min later,
the reaction was terminated by addition of 1 ml of ice-
cold methanol. PGD\(_2\) formed in the reaction mixture
was partially purified and determined as described above. The
inhibitory effects of test drugs were expressed as the per-
cent reduction in the amount of PGD\(_2\) formed compared
with that determined in the presence of the vehicle only.

PGE synthase (PGH-PGE isomerase) in sheep vesiculo-
gland microsomes was assayed with a PGH-PGE
isomerase activity kit (Nova Med, Jerusalem, Israel). The
reaction mixture containing 50 mM Tris-HCl (pH 7.5),
100 mM NaCl, 0.1 mM EDTA, 0.5 mM reduced
succinate and 30 μg/ml sheep vesicular gland micro-
somes was preincubated with the test drug or the vehicle
at 37°C for 10 min. The reaction was started by the addi-
tion of 2.8 μM (final concentration) PGH\(_2\), and 1 min
later, the reaction was terminated by addition of 0.75 ml of ice-cold methanol. PGE$_2$ formed in the reaction mixture was partially purified and determined as described above.

Statistics
The results were expressed as the mean±S.E.M. The data were analyzed by one-way analysis of variance (ANOVA) or the Kruskal-Wallis test. To determine the significance of differences among the groups, Dunnett’s or Scheffe’s multiple comparison tests were used. The 50% inhibition dose was estimated by the median effect analysis (15).

RESULTS

Effects of HQL-79 on histamine- and 5-HT-induced contraction of isolated guinea pig ileum

As shown in Fig. 1A, HQL-79, epinastine and ketotifen inhibited the histamine-induced contraction of isolated guinea pig ileum in a concentration-dependent manner. The concentrations of HQL-79, epinastine and ketotifen required to inhibit the reaction by 50% (IC$_{50}$ values) were estimated to be 50, 8.0 and 3.4 nM, respectively. The antihistaminic effect of HQL-79 in the guinea pig ileum was less potent than those of epinastine and ketotifen. HQL-79 was also less effective against 5-HT-

![Figure 1A](image1.png)

![Figure 1B](image2.png)

Fig. 1. Effects of HQL-79, epinastine and ketotifen on histamine- (A) and 5-HT- (B) induced contraction of isolated guinea pig ileum. The preparation was preincubated with test drugs at 10 min before the addition of histamine or 5-HT. Each point represents the mean±S.E.M. of 7 or 8 ileal segments from 4 guinea pigs. ●: HQL-79, △: Epinastine, ○: Ketotifen.
induced contraction of the ileal muscle than epinastine and ketotifen. The IC₅₀ values of HQL-79, epinastine and ketotifen were estimated to be 130, 23 and 2.1 nM, respectively (Fig. 1B).

Effects of HQL-79 on histamine- and 5-HT-induced contraction of isolated guinea pig trachea

As shown in Fig. 2A, HQL-79 and the reference drugs inhibited histamine-induced contraction of isolated guinea pig trachea in a concentration-dependent manner. The IC₅₀ values of HQL-79, epinastine and ketotifen were estimated to be 35, 12 and 3.7 nM, respectively. These drugs also inhibited 5-HT-induced tracheal contraction. The IC₅₀ values of HQL-79, epinastine and ketotifen were estimated to be 520, 47 and 270 nM, respectively (Fig. 2B). HQL-79 was less potent in inhibiting tracheal contraction induced by histamine and by 5-HT than epinastine and ketotifen.

Effect of HQL-79 on histamine-induced cutaneous reaction in rats

Orally administered HQL-79 dose-dependently inhibit-
Fig. 3. Effects of HQL-79, epinastine and ketotifen on histamine-induced cutaneous reaction in rats. Test drugs were given orally 1 hr before histamine injection. Each point represents the mean ± S.E.M. of 6 or 7 rats. *P < 0.05, **P < 0.01 vs vehicle-treated group (Scheffe’s test). ●: HQL-79, △: Epinastine, ○: Ketotifen.

ed histamine-induced cutaneous vascular permeability in rats; a significant effect was observed at doses of 0.01–1 mg/kg (Fig. 3). Ketotifen significantly inhibited the reaction at doses of 1–10 mg/kg. A significant effect of epinastine was observed only at 10 mg/kg. The doses of HQL-79, epinastine and ketotifen required to inhibit the reaction by 50% (ID₅₀ values) were estimated to be 0.03, 5 and 0.8 mg/kg, respectively. HQL-79 showed substan-

Fig. 4. Effects of HQL-79, epinastine and ketotifen on histamine-induced bronchoconstriction in guinea pigs. Test drugs were given orally 2 hr before histamine injection. Each point represents the mean ± S.E.M. of 6–8 guinea pigs. *P < 0.05, **P < 0.01 vs vehicle-treated group (Scheffe’s test). ●: HQL-79, △: Epinastine, ○: Ketotifen.
tially higher potency in inhibiting the histamine-induced skin reaction in rats than the reference drugs, although the in vitro antihistaminic activities of HQL-79 were less potent than those of epinastine and ketotifen (Figs. 1 and 2).

**Effects of HQL-79 on spasmoden-induced bronchoconstrictions in guinea pigs**

As shown in Fig. 4, orally administered HQL-79 significantly inhibited histamine-induced bronchoconstriction in anesthetized guinea pigs at doses of 0.01–0.03 mg/kg. Significant effects of epinastine and ketotifen were observed at doses of 0.30 and 0.003–0.03 mg/kg, respectively (Fig. 4). The ID$_{50}$ values of HQL-79, epinastine and ketotifen were estimated to be 0.009, 0.1 and 0.002 mg/kg, respectively. The inhibitory effect of HQL-79 on histamine-induced bronchoconstriction was remarkably strong, taking the less potent antihistaminic activities in vitro than those of the reference drugs (Figs. 1 and 2) into consideration.

Orally administered HQL-79 also inhibited 5-HT-induced bronchoconstriction at doses of 0.3–3 mg/kg (Fig. 5). The estimated ID$_{50}$ value (0.39 mg/kg) was comparable to those of epinastine and ketotifen (ID$_{50}$ values: 0.22 and 0.19 mg/kg, respectively). On the other hand, HQL-79 (3 mg/kg, i.v.) showed no significant inhibitory effects on the bronchoconstrictions induced by the other spasmodens, ACh, LTD$_4$, BK, NKA, PAF, PGD$_2$ and SP (data not shown).

**Effects of HQL-79 on antigen-induced release of LTC$_4$, LT $\alpha$ and histamine from the lung tissues of actively sensitized guinea pigs**

HQL-79 inhibited the antigen-induced release of LT $\alpha$ and LTC$_4$ from the chopped lung tissues of actively sensitized guinea pigs in a concentration-dependent manner, and a significant effect was observed at concentrations of 30–300 $\mu$M (Fig. 6: A and B). Significant effects of epinastine on the release of LT $\alpha$ and LTC$_4$ were observed at concentrations of 100 and 300 $\mu$M, respectively. The IC$_{50}$ values of HQL-79 and epinastine for the LT $\alpha$ release inhibition were estimated to be 106 and 99.1 $\mu$M, and those for the LTC$_4$ release inhibition were 67.4 and 82.0 $\mu$M, respectively. The inhibitory action of HQL-79 on histamine release was observed only at 300 $\mu$M (Fig. 6C).

**Effects of HQL-79 on antigen-induced release of PGD$_2$ and PGE$_2$ from the lung tissues of actively sensitized guinea pigs**

HQL-79 and epinastine inhibited the antigen-induced release of PGD$_2$ from the chopped lung tissues of actively sensitized guinea pigs in a concentration-dependent manner (Fig. 7). The IC$_{50}$ values of HQL-79 and epinastine were estimated to be 83 and 40 $\mu$M, respectively. Ketotifen inhibited the PGD$_2$ release only at 300 $\mu$M. Interestingly, the release of PGE$_2$ was significantly enhanced by HQL-79 at concentrations of 100–300 $\mu$M (Fig. 7). In contrast, epinastine inhibited the PGE$_2$

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**Fig. 5.** Effects of HQL-79, epinastine and ketotifen on 5-HT-induced bronchoconstriction in guinea pigs. Test drugs were given orally 2 hr before 5-HT injection. Each point represents the mean±S.E.M. of 6–8 guinea pigs. *P<0.05, **P<0.01 vs vehicle-treated group (Scheffe's test). ●: HQL-79, △: Epinastine, ○: Ketotifen.
release. Ketotifen did not affect the PGE₂ release.

**Effects of HQL-79 on LTBA, LTC₄, PGD₂ and PGE₂ contents in the lungs of repeatedly antigen-exposed guinea pigs**

Effects of chronic administration of HQL-79 on the production of the arachidonic acid metabolites were examined in guinea pigs repeatedly exposed to antigen. The results are summarized in Table 1. Chronic treatment with HQL-79 (10 or 30 mg/kg, 2 weeks) dose-dependently reduced PGD₂ and enhanced PGE₂ contents in the lungs, whereas the LTBA and LTC₄ contents were not

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**Fig. 6.** Effects of HQL-79, epinastine and ketotifen on antigen-induced release of LTBA (A), LTC₄ (B) and histamine (C) from the lung tissues of actively sensitized guinea pigs. The tissues were preincubated with test drugs at 60 min before antigen addition. Each point represents the mean ± S.E.M. of 5 or 6 preparations from 7 guinea pigs. *P < 0.05, **P < 0.01 vs vehicle-treated group (Scheffe’s test). ●: HQL-79, △: Epinastine, ○: Ketotifen.
Fig. 7. Effects of HQL-79, epinastine and ketotifen on antigen-induced release of PGD₂ (left) and PGE₂ (right) from the lung tissues of actively sensitized guinea pigs. The tissues were preincubated with test drugs at 60 min before antigen addition. Each point represents the mean ± S.E.M. of 5 or 6 preparations from 7 guinea pigs. *P < 0.05, **P < 0.01 vs vehicle-treated group (Scheffe's test). ●: HQL-79, △: Epinastine, ○: Ketotifen.

Table 1. Effects of HQL-79 on LTB₄, LTC₄, PGD₂ and PGE₂ contents in the lungs of antigen-exposed guinea pigs

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>LTB₄ (pg/g tissue)</th>
<th>LTC₄ (pg/g tissue)</th>
<th>PGD₂ (ng/g tissue)</th>
<th>PGE₂ (ng/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>—</td>
<td>7</td>
<td>117.6±37.8</td>
<td>692.7±106.2</td>
<td>177.6±23.3</td>
<td>56.3±9.7</td>
</tr>
<tr>
<td>HQL-79</td>
<td>10</td>
<td>8</td>
<td>49.5±10.1</td>
<td>400.6±43.4</td>
<td>100.4±11.7**</td>
<td>93.1±8.3*</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>8</td>
<td>57.6±11.8</td>
<td>600.6±82.6</td>
<td>75.2±7.0**</td>
<td>101.0±6.5**</td>
</tr>
</tbody>
</table>

Actively sensitized guinea pigs were exposed to the antigen on days 8, 15 and 22. The lungs were removed from the animals 30 min after the final antigen exposure (day 22). HQL-79 was given orally 13 times from days 8 to 22. Each value represents the mean ± S.E.M. of 7 or 8 guinea pigs. *P < 0.05, **P < 0.01 vs vehicle-treated group (Scheffe's test).

significantly affected.

Effects of HQL-79 on PGD synthase and PGE synthase activities

To clarify the mechanisms for the differential effects of HQL-79 on PGD₂ and PGE₂ production, effects of HQL-79 on the enzyme activities of PGD synthase and PGE synthase, enzymes responsible for PGD₂ and PGE₂ biosynthesis, respectively, were examined. HQL-79 inhibited PGD synthase activities in mouse spleen cytosol fractions in a concentration-dependent manner (Fig. 8). The IC₅₀ value for PGD synthase inhibition was estimated to be 51.0 μM. None of the antiallergics epinastine, terfenadine, oxatomide and cetirizine, significantly inhibited the PGD synthase at 300 μM (Fig. 8). On the other hand, HQL-79 had no significant effect on PGE synthase in sheep vesicular gland microsomes (Table 2).

DISCUSSION

In the present study, the effects of the newly synthesized antiallergic drug HQL-79 on various chemical mediators and on chemical mediator release were investigated both in vitro and in vivo.

It was shown that orally administered HQL-79 markedly inhibited the histamine-induced cutaneous reaction in rats (ID₅₀: 0.03 mg/kg) and histamine-induced bronchoconstriction in guinea pigs (ID₅₀: 0.009 mg/kg). The
antihistaminic activities were much greater than those of epinastine and also greater in rats and somewhat less potent in guinea pigs than those of ketotifen. It is likely that the potent in vivo antihistaminic activities make significant contributions to the strong inhibitory effects on immediate hypersensitivity reactions such as passive cutaneous anaphylaxis in rats (ID$_{50}$: 0.16 mg/kg) and antigen-induced bronchoconstriction in guinea pigs (ID$_{50}$: 0.026 mg/kg) (8).

In contrast to the potent antihistaminic activities in vivo, the inhibitory effects of HQL-79 on the histamine-induced contractions of isolated guinea pig ileum and trachea were obviously less potent than those of the reference drugs. The discrepancy between the higher antihistaminic activities in vivo and the lower activities in vitro could be explained by much higher bioavailability of HQL-79 than those of the reference drugs or the existence of an active metabolite of HQL-79 with higher in vitro antihistaminic activities than those of HQL-79 itself and the reference drugs. The maximum plasma level (C$_{\text{max}}$) of HQL-79 in rats following oral administration at a dose of 3 mg/kg HQL-79 was 232 µg/ml (K. Watanabe et al., unpublished result), which was not so high compared to the C$_{\text{max}}$ of epinastine in rats (382.5 µg/ml) following oral administration at a dose of 5 mg/kg epinastine (a company report, Boehringer Ingelheim Japan). Therefore, it was considered that an active metabolite of HQL-79 might contribute to the potent in vivo antihistaminic activities of HQL-79. We have analyzed the plasma obtained from rats that received oral administration of HQL-79. However, we have found no such active metabolite yet. Further investigations on the pharmacokinetics of HQL-79 will be required to elucidate the mechanisms for the higher in vivo antihistaminic activities.

HQL-79 inhibited histamine- and 5-HT-induced bronchoconstriction in guinea pigs. However, it did not affect bronchoconstrictions induced by the other spasmodens, used in this study ACh, LTD$_4$, BK, NKA, PAF, PGD$_2$ and SP. Epinastine and ketotifen have been reported to inhibit BK-induced bronchoconstriction in guinea pigs (16). Oxatomide inhibited LTD$_4$-induced guinea pig tracheal contraction (17). Antagonizing effects of HQL-79 on bronchial spasmodens were specific to histamine and 5-HT, compared to those reported in the histamine H$_1$-blocking antiallergics.

HQL-79 and epinastine inhibited the anaphylactic

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**Table 2. Effects of HQL-79 on PGE synthase activities in sheep vesicular gland microsomes**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Enzyme</th>
<th>Concentration (µM)</th>
<th>n</th>
<th>PGE$_2$ formed (ng/tube)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>77.5 ± 4.3</td>
</tr>
<tr>
<td>Vehicle</td>
<td>+</td>
<td>-</td>
<td>5</td>
<td>121.7 ± 8.4</td>
</tr>
<tr>
<td>HQL-79</td>
<td>+</td>
<td>10</td>
<td>5</td>
<td>125.0 ± 7.3</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>30</td>
<td>5</td>
<td>119.4 ± 14.1</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>100</td>
<td>5</td>
<td>137.3 ± 2.5</td>
</tr>
</tbody>
</table>

The microsomes were preincubated with HQL-79 or vehicle at 10 min before PGH$_2$ addition. Each value represents the mean±S.E.M. of 5 preparations.

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**Fig. 8.** Effects of HQL-79 and various antiallergic drugs on PGD synthase activities in mouse spleen. The preparation was preincubated with test drugs at 10 min before PGH$_2$ addition. Each point represents the mean±S.E.M. of 5 preparations.

**P < 0.01 vs vehicle-treated group (Dunnett's test). •: HQL-79, △: Epinastine, ○: Cetirizine, ▲: Oxatomide, □: Terfenadine.**
release of LTB₄ and LTC₄ from the chopped lung tissues of actively sensitized guinea pigs to a similar degree. On the other hand, the inhibitory action of HQL-79 on histamine release was less potent than that of epinastine. Many H₁-antihistaminic compounds, such as epinastine (2, 18), ketotifen (18), terfenadine (19), oxatomide (20) and levocabastine (21), have been found to suppress antigen- or calcium ionophore-induced release of chemical mediators. It has been suggested that the inhibitory effects of the antiallergics on chemical mediator release could be ascribed to the inhibition of cellular Ca²⁺ uptake (22, 23) and the enhancement of the cellular cyclic AMP level (9, 19, 21). In addition, oxatomide are known to inhibit 5-lipoxygenase (24). HQL-79 did not inhibit 5-lipoxygenase activities in rat basophil leukemia cells (data not shown). It is necessary to examine the effects of HQL-79 on the second messengers to clarify the mechanisms for chemical mediator release inhibition.

PGD₂, the major prostanoid metabolite produced by activated mast cells, is a potent bronchoconstricting mediator (25). PGD₂ is also known to activate isolated human eosinophils (26). In contrast, PGE₂ has been characterized as a bronchoprotective, anti-inflammatory and immunomodulatory prostaglandin (27, 28). Inhaled PGE₂ has been shown to protect against antigen- and exercise-induced bronchoconstriction in asthmatic subjects (29, 30). It has been therefore suggested that drugs that stimulate production of endogenous PGE₂ could act as antiasthmatic agents (31). HQL-79 inhibited antigen-induced release of PGD₂ and stimulated PGE₂ release from guine pig lung tissues in vitro. Moreover, the chronic treatment with HQL-79 clearly decreased the PGD₂ and increased the PGE₂ contents in the lungs of antigen-exposed guinea pigs. In a similar guinea pig asthmatic model, HQL-79 dose-depently inhibited antigen-induced airway eosinophilia (8). These findings suggest that the effects of HQL-79 on PGD₂ and PGE₂ production in the lungs contribute to the inhibitory effect on the airway inflammation in guinea pigs.

The chronic administration of HQL-79 obviously affected the PGD₂ and PGE₂ contents in the lungs of antigen-exposed guinea pigs. On the other hand, the LTB₄ and LTC₄ contents were not significantly altered by HQL-79, although HQL-79 inhibited antigen-induced LTB₄ and LTC₄ release from excised guinea pig lung tissues in vitro. The results in the in vivo experiment suggest that the chronic administration of HQL-79 modulated the synthesis of the PGs but did not affect the synthesis of the LTs. In the current experimental system, we could not detect changes in the release of the LTs from mast cells to the surrounding tissues, even though HQL-79 had inhibited LT release in the guinea pig lungs in vivo, since we determined the total amounts of the chemical mediators in whole lungs.

Both PGD₂ and PGE₂ are synthesized from the PG endoperoxide PGH₂, a key intermediate in PG biosynthesis. PGD synthase (PGH-PGD isomerase) and PGE synthase (PGH-PGE isomerase) are the enzymes responsible for PGD₂ and PGE₂ biosynthesis, respectively. To clarify the mechanisms for the effects of HQL-79 on PGD₂ and PGE₂ production, we have investigated the effects of the compound on these enzymes. HQL-79 clearly inhibited the PGD synthase in mouse spleen. Epinastine, terfenadine, oxatomide and cetirizine did not inhibit the PGD synthase, although these H₁-antihistamines have been reported to inhibit PGD₂ release from mast cells in vitro (24, 32). On the other hand, no significant effect of HQL-79 on the PGE synthase in sheep vesicular microsomes have been detected. Since both PGD₂ and PGE₂ are produced by the isomerization of the endoperoxide group of PGH₂, specific blockage of the isomerization of the 9-hydroxy-11-keto structure of PGD₂ might result in further isomerization of the 9-keto-11-hydroxy structure of PGE₂.

In conclusion, HQL-79 was shown to have potent antihistaminic- and anti-5-HT activities and inhibitory effects on chemical mediator release. In particular, the inhibitory effect on PGD synthase and the stimulatory effect on cellular PGE₂ production are conspicuous pharmacological features of HQL-79. It is suggested that these effects could be related to the inhibitory actions of HQL-79 on the antigen-induced immediate hypersensitivity reactions and the asthmatic late-phase inflammatory responses in various experimental models.

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