In Vitro Hepatic and Skin Metabolism of Capsaicin

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ABSTRACT:

On the basis of the ability of capsaicin to activate the transient receptor potential vanilloid 1 receptor (TRPV1) expressed in nociceptive sensory neurons, topical and injectable high-concentration formulations are being developed as potential treatments for various pain syndromes. As much of the published literature on capsaicin is based on pepper extracts, which are typically a mixture of capsaicin and other capsaicinoids (including norhydrocapsaicin, dihydrocapsaicin, homocapsaicin and homodihydrocapsaicin), the purpose of this investigation was to study the in vitro metabolism of pure capsaicin. The metabolism of capsaicin was similar in human, rat, and dog microsomes and S9 fractions.

In these assays, three major metabolites were detected and identified as 16-hydroxycapsaicin, 17-hydroxycapsaicin, and 16,17-dehydrocapsaicin. In addition to these three metabolites, rat microsomes and S9 fractions also produced vanillylamine and vanillin. Biotransformation of capsaicin was slow in human skin in vitro, with the majority of the applied capsaicin remaining unchanged and a small fraction being metabolized to vanillylamine and vanillic acid. These data suggest that the metabolism of capsaicin by cytochrome P450 enzymes in skin is minimal, relative to hepatic metabolism.

Capsaicin is the most abundant pungent molecule produced by pepper plants and thereby represents an important ingredient in spicy foods consumed throughout the world. The capsaicin content of peppers ranges from 0.1 to 2.5 mg/g (Parrish, 1996), and the resulting average human capsaicin consumption is on the order of 0.5 to 4 mg/kg/day (EC Scientific Committee on Food, 2002, http://ec.europa.eu/food/fs/sc/scf/out120_en.pdf). In addition to its extensive role as a food additive, there is also substantial human exposure to capsaicin in the form of nonprescription (in the United States) or prescription (in the European Union) topical analgesics, self-defense products (e.g., pepper spray), and oral herbal supplements.

Capsaicin is a highly selective agonist for the TRPV1 [formerly known as the vanilloid receptor 1 (VR1)]. TRPV1 is a ligand-gated, nonselective cation channel preferentially expressed on small-diameter sensory neurons, especially those nociceptors that specialize in the detection of painful or noxious sensations (C-fibers and to a lesser extent Aδ-fibers) (Caterina et al., 1997; Szallasi and Blumberg, 1999). The initial effect of capsaicin is the activation of TRPV1-expressing nociceptors, resulting in a burning sensation, hyperalgesia, allodynia, and erythema (Szallasi and Blumberg, 1999); these events are followed by a reversible defunctionalization of nociceptive sensory axons (Bley, 2004). Defunctionalization of hyperactive nociceptors is thought to underlie the pain relief that follows topical application or intra-articular injections of capsaicin (Bley, 2004).

Much of the published literature on capsaicin relates to extracts of capsaicin derived from peppers; these extracts are typically a mixture of capsaicin, norhydrocapsaicin, dihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin. The actual percentage of capsaicin and other capsaicinoids varies depending on the peppers used and method of extraction and can range from approximately 65% (USP grade natural capsaicin) to ≥99% (for synthetically pure capsaicin). In addition, extracts may contain chemical entities other than vanilloiold compounds. As a consequence of these variable impurity profiles, results obtained from metabolism studies with pure capsaicin may differ from results obtained with extracts.

Previous publications suggest that capsaicinoids are metabolized to a great extent by hepatic enzymes (Reilly and Yost, 2006). Early studies demonstrated that capsaicin is converted to metabolites via hydroxylation of the vanillyl ring moiety, leading to the hypothesis that capsaicin is activated by the liver mixed-function oxidase system to an electrophilic intermediate that is capable of covalently binding to hepatic proteins. Additionally, the alkyl side chain of capsaicin was also considered to be susceptible to enzymatic oxidation, giving rise to a hydroxylated metabolite at the terminal carbon of the side chain when incubated with NADPH and a liver S9 fraction. One-electron oxidation of capsaicin also plays a role in capsaicin metabolism. Liver cytochrome P450 2E1 (CYP2E1) activity has been shown to be responsible for conversion of capsaicin to the reactive phenoxy radical, which in turn can dimerize or bind to CYP2E1, thereby inactivating the enzyme (Surh and Lee, 1995). This mechanism may be responsible for the inhibition of further activation and the reported chemoprotective activity of capsaicin against some chemical carcinogens and mutagens (Surh and Lee, 1996). Nonoxidative hydrolysis has also been demonstrated in rat tissues at the acid-amide bond. The splitting of the side chain also occurs in vivo, which is believed to be

ABBREVIATIONS: TRPV1, transient receptor potential vanilloid 1 receptor; ACN, acetonitrile; LSC, liquid scintillation counting; HPLC, high-performance liquid chromatography; HBSS, Hanks’ balanced salt solution; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry.
the rate-limiting step in overall metabolism of the compound. Oxida-
tive deamination of the resulting vanillylamine produces the aromatic
aldehyde, vanillin, which in turn undergoes oxidation to vanillic acid
or reduction to vanillyl alcohol for excretion as a free form or as a
glucuronic acid conjugate (Surh and Lee, 1995). In addition to
CYP2E1, capsaicin can also be metabolized by CYP1A2, 1A1, 3A4,
2B6, 2C8, 2C9, and 2C19 (Reilly et al., 2003). Some of these
pathways also represent a detoxification process, as opposed to the
bioactivation pathways discussed earlier, resulting in reduction in
cytotoxicity (Reilly et al., 2003; Reilly and Yost, 2006).

The purpose of the presently reported studies was to explore rig-
ously the in vitro metabolism of highly pure capsaicin at physi-
ologically relevant concentrations, using liver microsomes and S9
fractions as well as fresh human skin. Liver microsomes and S9
fractions from rat, dog, and human were used for interspecies com-
parison of capsaicin metabolism. S9 fractions were used to detect in-
volved implication of any phase 2 metabolism.

Materials and Methods

Materials. Capsaicin (CAS number 404-86-4) was manufactured under
current good manufacturing practice conditions and had ≥99% purity. Al-
though there are two geometric isomers of capsaicin, only trans-capsaicin
occurs naturally (Cordell and Araujo 1993), and thus the term “capsaicin” is
used generically to refer to the trans-geometric isomer. [14C]Capsaicin (Fig. 1)
was synthesized by Chemsyn (Lenexa, KS) with specific activity of 54.0
mCi/mmol. Radiochemical purity, as well as purity of trans-capsaicin, was
≥98%. Vanillin and vanillylamine hydrochloride were purchased from Aldrich
Chemical Co. (Milwaukee, WI), and vanillic acid and vanillyl alcohol were
purchased from Fluka Chemical Corp. (Ronkonkoma, NY). Separately pooled
male rat, dog, and human hepatic microsomes and S9 fractions and fresh
human skin samples were obtained from In Vitro Technologies (Baltimore,
MD). Microsomes and S9 fractions were stored frozen at approximately
−70°C.

Microsomal and S9 Incubations. For the hepatic metabolism studies, rat,
dog, and human hepatic microsomes and S9 fractions were diluted with buffer
(0.1 M potassium phosphate buffer, pH 7.4, containing 1 mM EDTA) to give
final protein concentrations of 1 mg/ml. Microsomal and S9 fractions suspensions
were then preincubated with a NADPH-solution at 37°C for 5 min in buffer.
Incubations were initiated by the addition of 10 μl of a 100-fold
centrifuged solution of [14C]capsaicin in dimethyl sulfoxide to give final
concentrations of 1 and 10 μM [14C]capsaicin. Control incubations were
performed at 1 and 10 μM [14C]capsaicin for 0 and 30 min in the absence of
the NADPH and medium. The final incubation volume was 1 ml, and all
incubations were conducted in duplicate. The amounts of radiolabeled test
article were approximately 120,000 dpm/ml (1 μM [14C]capsaicin) and
200,000 dpm/ml (10 μM [14C]capsaicin). The final concentration of dimethyl
sulfoxide in the incubation samples was approximately 1% (v/v). Incubation
reactions were terminated at 0, 5, 10, 20, and 30 min by the addition of 1 ml of
acetoniitrile (ACN), followed by vortex mixing. Precipitated microsomal and
S9 fraction protein was removed by centrifugation (1400g for 15 min, at
approximately 4°C) and the supernatants were analyzed by liquid scintillation
counting (LSC) and HPLC analysis.

Skin Metabolism. For skin metabolism, all incubations were conducted at
37 ± 1°C, 95% air/5% CO2, and saturating humidity. The medium used in
this study was Hanks’ balanced salt solution (HBSS) supplemented with 1% bovine
serum albumin. Fresh skin from the abdominal region of one female human donor
was obtained and cut with a dermatome to a thickness of 250 to 350 μm. Skin
disks (24 disks, 14–18 mm in diameter) were prepared from the skin using a
cork-boring tool and stored in supplemented HBSS kept on wet ice until incuba-
tion. Skin disks were transferred to 12-well tissue culture plates, with each well
containing 1 ml of a solution of 0, 1, 3, or 10 μM [14C]capsaicin (up to 200,000
dpm/ml). Skin disks from each concentration were incubated on an orbital shaker
for 20 h. After the 20-h incubation, the skin disks were harvested into individually
labeled vials. An equal volume of ACN was added to the medium samples. The
samples were then harvested into individually labeled cryovials and stored at −70°C.
The medium samples were then returned to −70°C storage until analysis. The
control and incubation medium samples were analyzed by HPLC directly. Before HPLC analysis, duplicate
aliquots of each sample were then taken and analyzed by LSC to determine the initial
concentration of radioactivity in each sample. The human skin disks were pooled
by concentration. The skin disks were cut into small pieces and then ground using
a Potter-Elvehjem probe. To the ground samples, 5 ml of ACN was added and
mixed for 10 min, and then the samples were transferred to centrifuge tubes. The
samples were then vortexed, sonicated for approximately 15 min, and centrifuged
at approximately 3300 rpm at room temperature, and the supernatant fractions
were decanted to separate tubes. This extraction procedure was repeated, and the
corresponding supernatant fractions were combined. The supernatant fractions
were dried under nitrogen and reconstituted in 0.1% acetic acid in water-ACN
(50:50). Duplicate aliquots were analyzed by LSC to determine the radioactivity
concentration in the extracts. Incubated media and human skin samples were
analyzed using HPLC with radiochemical detection.

Medium control samples were included to evaluate the chemical degrada-
tion of [14C]capsaicin in the absence of a metabolically active system.
[14C]Capsaicin at 1, 3, and 10 μM (1 ml, up to 200,000 dpm/ml) was incubated in
12-well tissue culture plates without skin disks on an orbital shaker for 0 or
20 h. For the 0-h sampling, an equal volume of ACN was added to the medium
control samples. Two 100-μl aliquots of the medium control samples were
taken for analysis by liquid scintillation counting. The remaining medium
control samples were harvested into individually labeled vials. The medium
control samples were stored at −70°C until analysis. After incubation for 20 h,
an equal volume of ACN was added to the medium control samples. The
samples were harvested into individually labeled cryovials and stored at −70°C.
The medium control samples were then returned to −70°C storage until analysis.
For analysis of the medium control samples, liquid scintillation fluid was added
the aliquot of each medium sample, and then the sample was placed in
the dark at ambient temperature for at least 24 h to allow chemiluminescence
to decay. The samples were analyzed using LSC.

Viability control samples were included to assess the mitochondrial function
of representative skin disks. Human skin disks were evaluated for viability
after incubation for 0 or 20 h. After each incubation time point, the medium
from each viability control well was removed and replaced with supplemented
HBSS medium containing tetrazolium dye 3-[4,5-dimethylthiazol-2-yl]-2,5-
diphenyltetrazolium bromide (MTT) (0.5 mg/ml), and the skin samples were
incubated for an additional 3 h. After the 3-h incubation, the MTT dissolving
solution was removed, and the skin samples were extracted with acidified
isopropanol at 4°C for 8 to 12 h to extract the MTT formazan. Samples were
analyzed within 24 h of the start of extraction by spectrophotometry at 570 nm
(with reference to 690 nm) on a plate reader.

Radio-HPLC Analysis. Samples generated from microsomal, S9, and skin
incubations were analyzed by radio-HPLC using a MetChem Inertsil 5-μm
ODS-2 column (4.6 × 250 mm; 25°C column temperature) and radioactive
detector (Packard 500 series), with Ultima-Flo M as the scintillation cocktail.
The mobile phase was a gradient with A (0.1% acetic acid in reverse osmosis
water) and B (ACN). The flow rate was 1 ml/min. The initial liquid chroma-
tography conditions were held at 2% B for 7 min, followed by a linear gradient
from 2 to 90% B over 33 min and back to 2% B over 5 min. The scintillation
cocktail flow rate was 3 ml/min.

LC/MS Analysis. Microsomes and S9 incubation extracts were first ana-
lyzed using a fill-scan LC/MS analysis with a MetChem Inertsil 5 μm ODS-2
(250 mm × 4.6 mm) reversed-phase HPLC column eluted at a flow of rate of

Fig. 1. Structure of capsaicin. *, position of the radiolabel.
1 ml/min with a mobile phase consisting of 0.02% acetic acid in reverse osmosis water and acetonitrile and a Micromass Quattro II with an ESP Z-Spray source mass spectrometer. The column temperature was maintained at 25°C. During analysis after passing through the column switcher, the HPLC column effluent was split with approximately 30% of the flow diverted to the mass spectrometer and 70% to the radioactivity detector. To minimize contamination of the mass spectrometer source, the first 3.5 min of each run was diverted to waste using the switching valve. The mass spectrometer was equipped with an electrospray ionization source and was set to scan for negative ions in the range of m/z 25 to 650. The cone voltage was set at 22 V, z

**Results**

**[14C]Capsaicin Metabolism in Rat, Dog, and Human Hepatic Microsomes and S9 Fractions.** The radioisotopy of [14C]capsaicin ranged from approximately 97 to 98%. Identification of capsaicin metabolites was based on HPLC coelution with authentic standards and on mass spectral analysis of metabolites in rat, dog, and human microsomes and S9 fractions. Both negative and positive ion electrospray LC/MS were used to analyze metabolites and standards.  

**Biotransformation of [14C]Capsaicin in Rat Hepatic Microsomes and S9 Fractions.** In rat microsomes, [14C]capsaicin was metabolized rapidly. In control incubations, approximately 95% of the radioactivity was in the form of [14C]capsaicin. After 5 min of incubation with [14C]capsaicin at 1 and 10 μM, only 2.24 and 51.0% of the radioactivity was associated with [14C]capsaicin. [14C]Capsaicin was completely consumed in 1 and 10 μM incubations at 10 and 20 min, respectively. As many as 10 metabolites were detected in incubations at both concentrations. However, some of them accounted for very little of the radioactivity and were not characterized. Major metabolites (Tables 1 and 2) included vanillin, vanillylamine, 16-hydroxycapsaicin, and 16,17-dehydrocapsaicin. 16-Hydroxycapsaicin was the most abundant metabolite, accounting for 47.0 to 52.5 and 21.3 to 49.4% of the radioactivity in samples at 1 and 10 μM, respectively. 16,17-Dehydrocapsaicin accounted for 18.8% of the radioactivity in the sample at 5 min at 1 μM, whereas at 10 μM 16,17-dehydrocapsaicin accounted for 4.65 to 18.9% of the radioactivity in the sample from 5 to 20 min. The transient nature of this metabolite suggests that it was further metabolized during the incubations.

**Biotransformation of [14C]Capsaicin in Dog Hepatic Microsomes and S9 Fractions.** Metabolism of [14C]capsaicin in rat S9 fractions was slower than that in microsomes. Unchanged [14C]capsaicin detected at 10 min accounted for 13.4% of the radioactivity in samples at the 1 μM concentration and by 20 min, no unchanged parent drug was detected. At the 10 μM concentration, unchanged parent drug accounted for 16.9% of the radioactivity in sample at 30 min. As many as seven metabolites were detected in S9 fractions. Vanillylamine, 16-hydroxycapsaicin, and 16,17-dehydrocapsaicin were the major metabolites at both concentrations. 16-Hydroxycapsaicin accounted for 28.6 to 52.4 and 7.23 to 34.7% of the radioactivity in sample in 1 and 10 μM concentrations, respectively, from 5 to 30 min. 16,17-Dehydrocapsaicin accounted for 5.91 to 20.7 and 7.17 to 15.5% of the radioactivity in samples in 1 and 10 μM from 5 to 20 and 5 to 30 min, respectively. All remaining unknown metabolites were each ≤10% of the radioactivity in the sample.

**Biotransformation of [14C]Capsaicin in Human Hepatic Microsomes and S9 Fractions.** Metabolism of [14C]capsaicin in dog microsomes and S9 fractions was less extensive than in equivalent rat fractions. Four metabolites (Tables 1 and 2) were detected in microsomal incubations in addition to unchanged [14C]capsaicin. By 30 min, unchanged parent drug accounted for 23.8 and 37.7% of the radioactivity in the samples in 1 and 10 μM concentrations, respectively. 16-Hydroxycapsaicin was the major metabolite in microsomal incubations and accounted for 5.68 to 43.7 and 3.10 to 28.3% of the radioactivity in samples. 17-Hydroxycapsaicin was also a significant metabolite. Additional metabolites detected in dog microsomes and S9 fractions included hydroxycapsaicin (determination of the location of hydroxyl group on the alkyl chain was not possible by LC/MS/MS) and 16,17-dehydrocapsaicin.

**Biotransformation of [14C]Capsaicin in Dog Hepatic Microsomes and S9 Fractions.** Metabolism of [14C]capsaicin in dog microsomes and S9 fractions was less extensive than in equivalent rat fractions. Four metabolites (Tables 1 and 2) were detected in microsomal incubations in addition to unchanged [14C]capsaicin. By 30 min, unchanged parent drug accounted for 23.8 and 37.7% of the radioactivity in the samples in 1 and 10 μM concentrations, respectively. 16-Hydroxycapsaicin was the major metabolite in microsomal incubations and accounted for 5.68 to 43.7 and 3.10 to 28.3% of the radioactivity in samples. 17-Hydroxycapsaicin was also a significant metabolite. Additional metabolites detected in dog microsomes and S9 fractions included hydroxycapsaicin (determination of the location of hydroxyl group on the alkyl chain was not possible by LC/MS/MS) and 16,17-dehydrocapsaicin.

**Biotransformation of [14C]Capsaicin in Human Hepatic Microsomes and S9 Fractions.** Metabolism of [14C]capsaicin was rapidly metabolized by human microsomal and S9 fractions. As many as five metabolites were detected. At both the 1 and 10 μM concentrations (Tables 1 and 2), 16-hydroxycapsaicin and 17-hydroxycapsaicin were
Percentage of radioactivity in incubation samples of rat, dog, and human hepatic microsomes and S9 fractions at 1 mg of protein/ml with [14C]-capsaicin at 10 μM for 0, 5, 10, 20, and 30 min

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Time Point</th>
<th>Vanillylamine</th>
<th>Vanillin</th>
<th>16-HC</th>
<th>17-HC</th>
<th>16,17-DHC</th>
<th>Capsaicin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>M</td>
<td>S9</td>
<td>M</td>
<td>S9</td>
<td>M</td>
<td>S9</td>
</tr>
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<td>N.D.</td>
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<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.52</td>
<td>4.44</td>
<td>N.D.</td>
<td>21.3</td>
<td>7.23</td>
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<tr>
<td></td>
<td>10</td>
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<td>15.3</td>
<td>8.78</td>
<td>1.22</td>
<td>49.4</td>
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<tr>
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<td>30</td>
<td>9.69</td>
<td>17.8</td>
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<td>5.05</td>
<td>42.8</td>
<td>34.7</td>
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<tr>
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<tr>
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<td>N.D.</td>
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</tr>
<tr>
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<td>20.6</td>
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6-HC, 16-hydroxycapsaicin; 17-HC, 17-hydroxycapsaicin; 16,17-DHC, 16,17-dehydrocapsaicin; M, liver microsomes; N.D., not detected.

Discussion

Although capsaicin is widely consumed orally throughout the world, limited information has appeared regarding its metabolism by liver enzymes. Moreover, there has been no published information on specific metabolism by skin. Therefore, the data presented here represent the first detailed and systematic characterization of capsaicin that would be applicable to the pure form used in the topical and injectable medical products in clinical development.

In rats, the profile of metabolites of [14C]-capsaicin was similar in both microsomal and S9 fractions at both concentrations. However, the extent of metabolism was reduced at the higher concentration, suggesting that the rates of metabolism of [14C]-capsaicin are saturable. At both concentrations in medium control, [14C]-capsaicin was recovered quantitatively, whereas in S9 control samples without NADPH, recovery of [14C]-capsaicin was approximately 80%. In this system the rest of the radioactivity was found to be vanillylamine. This result indicates that hydrolysis of the amide bond of capsaicin may be in part mediated by amidases in the soluble fraction of liver. As was observed in the rat, metabolism of [14C]-capsaicin in dog S9 fractions was qualitatively similar but less extensive than in microsomes. Biotransformation in both human microsomes and S9 fractions was qualitatively similar at both concentrations, although metabolism of [14C]-capsaicin was less extensive in S9 fractions than in microsomes.

After incubation with rat, dog, and human microsomes and S9 fractions, capsaicin was metabolized to several products. As proposed in the biotransformation pathway shown in Fig. 3, capsaicin was converted in microsomes and S9 fractions to at least five primary metabolites. A number of side chain-hydroxylated metabolites were detected including two major metabolites, 16-hydroxycapsaicin and 17-hydroxycapsaicin. Oxidation of capsaicin or loss of water from the hydroxylated metabolites generated the amide bond of capsaicin produced vanillylamine. Further oxidation of vanillylamine generated a secondary metabolite, vanillin. Proposed generation of vanillylamine from capsaicin and subsequent metabolism to vanillin is similar to the metabolic pathway of dihydrocapsaicin suggested by Kawada and Iwai (1985). Additional metabolites detected in dog microsomes and S9 fraction included hydroxydihydrocapsaicin.

The extent of metabolism was similar in dog microsomes and S9 fractions but was slower than that observed in human and rat. All three major metabolites detected in human were also present in dog microsomal and S9 fractions samples. Neither vanillylamine nor vanillin was detected in rat. However, in dog a hydroxydihydrocapsaicin was generated that was not present in either rat or human. In rat microsomes and S9 fractions, high levels of vanillylamine and vanillin were detected, indicating high hydrolytic enzyme activities. In rat, two major metabolites, 16-hydroxycapsaicin and 16,17-dehydrocapsaicin, were detected. A third major metabolite, 17-hydroxydihydrocapsaicin, which was detected in human and dog, was present in rat samples at lower levels.

Biotransformation of [14C]-capsaicin was rapid in rat microsomal and S9 fraction incubations compared with dog and human incubations. In human, the rate of formation of the metabolites was faster than in dog. Generally, the rate of metabolism in microsomes was faster than in S9 fractions. For all species the metabolism of [14C]-capsaicin was slower than in microsomes. The major metabolites were 16-hydroxycapsaicin, 17-hydroxycapsaicin, and 16,17-dehydrocapsaicin.

Metabolism of [14C]-Capsaicin in Human Skin in Vitro. The viability controls indicated that the skin used in the incubations was viable. Conversion of MTT to MTT formazan was observed with skin disks incubated for 0 and 20 h. The optical densities of the MTT formazan extracts on average were 0.648 ± 0.237 and 0.740 ± 0.111 for skin disks incubated for 0 and 20 h, respectively. In control incubations for 0 or 20 h with 1, 3, and 10 μM [14C]-capsaicin, 99 to 100% of the radioactivity consisted of unchanged capsaicin, indicating stability of the test article in control medium under the incubation conditions. During incubation with human skin, [14C]-capsaicin was metabolized slowly over 20 h. Two metabolites, vanillylamine and vanillic acid, were detected in incubation medium and skin samples at all concentrations, with the exception of vanillic acid, which was not detected in incubation medium at the 1 μM concentration (Fig. 2). In the incubation medium, capsaicin, vanillylamine, and vanillic acid accounted for mean values of 91.0 to 95.6, 4.37 to 8.77, and <0.15% of the radioactivity in the samples, respectively. In skin, capsaicin, vanillylamine, and vanillic acid accounted for 74.0 to 78.7, 12.9 to 16.1, and 10.3 to 13.6% of the radioactivity in the samples, respectively.
Saicin was less extensive at the 10 μM concentration compared with the 1 μM concentration, suggesting saturability of metabolism. The metabolism of capsaicin was similar in microsomes and S9 fractions from rat, dog, and human. In rat, dog, and human, three major metabolites were detected and identified as 16-hydroxycapsaicin, 17-hydroxycapsaicin, and 16,17-dehydrocapsaicin. These metabolites are previously reported by Reilly et al. (2003). However, other metabolites reported by Reilly et al. were not detected. These undetected metabolites include one of the two aliphatic dehydrogenation products (current study detected only 16,17-dehydrocapsaicin), two aromatic hydroxylation products, an O-demethylation product, an N-dehydrogenation product, and an N-dehydrogenation and ring oxygenation product. However, in the current study, rat microsomes and S9 fractions also produced vanillylamine and vanillin metabolites, which were not reported by Reilly et al. These differences could possibly be attributed to using a higher concentration of capsaicin (100 μM, as opposed to 1 and 10 μM in the current study) and a longer incubation time (60 min, as opposed to 30 min in the current study). It is possible that a higher capsaicin concentration, combined with a longer incubation time, may have changed some of the metabolic pathways, which resulted in formation of more metabolites as reported by Reilly et al. that were not observed in the current study. Vanillylamine and vanillin metabolites have been reported previously in rats after oral administration of dihydrocapsaicin by Donnerer et al. (1990) and Kawada and Iwai (1985). However, no vanillyl alcohol or vanillic acid was detected in our studies.

There are no published data on the oral bioavailability of capsaicin. However, evidence from a rat intestinal ex vivo study predicts that the molecule will be readily absorbed from the intestinal tract (Monsereensorn, 1980). Therefore, capsaicin ingested from food is probably well absorbed and subjected to significant first-pass hepatic metabolism. Although capsaicin consumption has been speculated to...
that the formation of vanillylamine and vanillic acid was due to skin metabolism. In this tissue, capsaicin was metabolized to vanillylamine and vanillic acid, although the majority of the sample radioactivity was associated with unchanged capsaicin. Both of these metabolites, vanillylamine and vanillic acid, are also metabolites generated from the common flavor vanilla (Odink et al., 1988; Suresh et al., 2003). The implication for topical capsaicin-containing analgesic products is that capsaicin can reach its peripheral target site intact and can reside at the site of action unchanged for a longer period of time. Additionally, a lack of oxidative metabolism in skin would indicate that there is lack of potential for formation of covalently bound toxic metabolites.

In summary, capsaicin is rapidly metabolized in vitro by hepatic enzymes from three species but not by human skin. These studies have not identified any unexpected metabolites, metabolic pathways, or safety concerns for oral or topical capsaicin exposure.

Fig. 4. Proposed biotransformation pathway for capsaicin in skin.

References


