EXPRESSION OF VANILLOID RECEPTOR-1 IN THE DUODENUM OF THE CAPSAICIN TREATED RAT

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Abstract

The aim of the presented investigation was to identify different vanilloid receptor-1 (VR1) immunoreactivity after application of low dose of capsaicin (0.5 mg/kg) for a prolonged period in the rat’s duodenum. Paraffin-embedded sections were processed for standard immunohistochemistry by the labelled streptavidin-biotin technique. The VR1 localisations were identified on the epithelial layers of the villi, in the Brunner’s glands, smooth muscles layer, and the neurons of the myenteric plexus of the duodenum. While VR1 immunoreactivity was identified in small quantities in the control group, VR1 expression was strong both in the experimental and in the vehicle treated group. These results indicate that prolonged administration of a low dose of capsaicin may not be sufficient to stimulate VR1. Also the vehicle additives, Tween 80 and 10% ethanol, which are used to solubilize capsaicin, may activate the protection mechanism in the mucosa epithelium and stimulate the capsaicin sensitive afferent neurons by VR1 to increase mucus secretion.

Key words: rats, capsaicin, vanilloid receptors, immunohistochemistry, duodenum.

Capsaicin (CAP), (8-methyl-N-vanillyl-6-nonenamide), the main ingredient of hot chilli peppers, has been used for the study of pain or the study of properties of a subpopulation of primary afferent nerves (42). CAP causes immediate and severe pain by exciting sensory neurons. The excitation of sensory neurons by CAP is mediated by the activation of CAP receptors (5, 32). After initial excitation, sensory neurons do not respond to CAP after application of repeated or high doses of CAP (7, 37). CAP also causes neurogenic inflammation mediated by the release of neuropeptides such as substance-P (SP) or calcitonin gene related peptide (CGRP) from sensory nerve endings with a loss of their sensory-afferent functions, and their ability to release sensory neuropeptides (13). On the other hand, Hudson et al. (17) reported that VR1 protein is up-regulated in undamaged neurons and down-regulated in damaged neurons.

The aim of the present investigation was to determine the localisation of VR1 in rat duodenum by immunohistochemistry and to identify different VR1 immunoreactivity after the application of a low dose of capsaicin for a prolonged period in the rat’s duodenum, which might provide more remarkable effects on the gastrointestinal system, taking into account that the adequate dose may be consumed by humans from eating a spicy meal.
Material and Methods

Animals. Thirty immature female Sprague-Dawley rats (21 d old) were used throughout the experiments. The rats were obtained from the Experimental Animals Breeding and Research Centre, Uludag University, Turkey. The animals were housed five per cage, in temperature controlled conditions of (20–24ºC), humidity (60–70%), and lighting (12 h light/dark cycle), and were provided with feed and water ad libitum. The experimental protocols were approved by the Animal Care and Use Committee of the Uludag University and were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Experimental protocol. The rats were divided at random into 3 groups of 10 animals each. The first group (control) remained without any treatment. The second group (experimental) received subcutaneous injection of CAP (Sigma Chemical Co.) (0.5mg/kg/d), prepared in a solvent consisting of 10% of ethanol, 10% of Tween 80, and 80% of distilled water, for 20 consecutive days, and the third group (vehicle treated) received an equal volume of the solvent in the same way used for CAP.

Following 20 d of capsaicin treatment, the animals were euthanised by the injection of sodium pentobarbital and the abdominal walls were opened. The proximal part of the duodenum was removed and fixed in alcoholic formaldehyde. Tissue samples were embedded in paraffin blocks according to routine histological procedures. Five micrometres thick sections were cut and immunostained for VR1 localisation (19).

Immunohistochemistry. Polyclonal rabbit VR1 primary antibody (R-130, Santa Cruz) raised against an amino acids 1-130 mapping at the N-terminus of VR1 of rat origin was used for the VR1 immunostaining. The standard Streptavidin Biotin Peroxidase complex technique was carried out by using the Histostain Plus Kit (Zymed). Briefly, the sections were deparaffinized, hydrated, and put into 0.05% non-immune serum blocking solution for 1 h at room temperature before application of the VR1 antibody. The sections were incubated overnight at 4ºC with 1:1 000 dilution of the VR1 antibodies. The sections were incubated with biotinylated secondary rabbit antibody for 10 min followed by streptavidin conjugated to horseradish peroxidase for 10 min at room temperature. Finally, 3,3'-diaminobenzidine (DAB) was used for colour development, and haematoxylin was used for counterstaining. Negative control slides processed without primary antibodies were included for each staining.

All the slides were coded so that the investigator was blinded to staining for each slide and graded them according to the following scale: - no staining, + slight, ++ medium, +++ strong.

Results

The vanilloid receptor-1 localisation. VR-1 was identified on the epithelial layers of the villi, and in the Brunner’s glands, smooth muscles, and neurons of the myenteric plexus of the duodenum (Fig. 1). Differences in the expression of VR1 immunoreaction between the groups in the mentioned regions are presented in Table 1. In the regions, VR1 labelling was found to be distributed intracytoplasmically.

Mucosa. In the control group, the VR1 activity was observed on some of the surface epithelial cells of the villi and the intensity of expression in these cells was quite slight. Additionally, in experimental and vehicle treated groups, VR1 immunoreactivity was identified in the cytoplasm of all surface epithelial cells of the villi and in these cells the labelling appeared to be diffuse (Fig 2). It was detected that VR1 immunoreaction intensity in these two groups was quite strong and there was not any intensity difference between groups. No immunoreactivity was detected in the crypts in all the groups.

Submucosa. VR1 immunoreaction in Brunner’s glands of the submucosa showed intracytoplasmic location. Slight VR1 immunoreactivity was observed in the control group and medium immunoreaction was noted in two other groups (Fig 3).

Muscle layer. No VR1 immunoreaction was detected in circular muscle layer in all groups; however, in longitudinal muscle layer, VR1 immunoreaction was strong in both experimental groups except for control group, which had medium VR1 immunoreaction intensity. In all the groups, strong VR1 immunoreaction was detected in the neurons of the myenteric plexus (Fig 4).

Besides, VR1 immunoreaction was detected in the endothelial cells lining blood vessels. While no VR1 immunoreaction was identified in blood vessels of control group, VR1 expression was strong in experimental group and medium one in the vehicle treated group.

Table 1

<table>
<thead>
<tr>
<th>Region</th>
<th>Duodenum</th>
<th>Control</th>
<th>Vehicle treated</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface epithelium</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Brunner’s glands</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Circular muscle layer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Longitudinal muscle layer</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Myenteric plexus</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Endothelial cell</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>

Intensity of reaction:
- no staining, + slight, ++ medium, +++ strong.
Numerous studies have shown the existence of capsaicin sensitivity neurons in the gastrointestinal tract (11, 31, 34). These neurons are primary afferents and several important physiological functions in the gastrointestinal tract have been attributed to them (9, 22). In recent years, it has been shown that the action of capsaicin on afferent neurons can be mediated through activation of specific receptors, namely VR1 (5). VR1, also known as TRPV1 receptors, located on sensory nerve endings termed nociceptors, are sensitive to a variety of stimuli, including capsaicin, changes in pH, and increased temperature that reaches the noxious range (5).

In the present study, we demonstrated the expression of VR1 in the rat duodenum in paraffin sections by immunohistochemical technique. We observed VR1 immunoreactivity in the cytoplasm of surface epithelial cells of the villi. Similar to our findings, several papers demonstrated the presence of VR1 in a human bronchial epithelial cell line (41), skin epidermal keratinocytes (18), urinary bladder epithelial cells (4, 26), and rat gastric mucosal cells (31).

Activation of vanilloid receptors on afferent nerves in the gastrointestinal tract, causes increased elimination of acid from the lumen as a result of vasodilatation (14, 27, 28), increased mucus output (20), and increased bicarbonate production (39). Kechagias et al. (24) reported that VR1 immunoreactivity in antral epithelial cells may indicate gastro protective effects of capsaicin. Moreover, Kato et al. (22) recently identified expression of VR1 in a cultured rat gastric epithelial cell line, and showed that VR1 played a protective role against cellular damage. However, it was reported that these effects of capsaicin depended on the period and dosage of the treatment and stimulation of primary afferents by short term administration of capsaicin that caused increased gastric mucosal blood flow (10). In contrast, Nozawa et al. (31) reported that the application of capsaicin for prolonged periods of time could have irreversible toxic effects leading to the loss of neurons; and also showed that a neurotoxic dose of capsaicin markedly reduced the immunoreactivity of VR1 in the rat stomach.

In the present study, the intensity of immunoreactivity was stronger in the epithelial layer of the other groups when compared with the control group. The lack of a significant difference between the vehicle treated group and the experimental group, suggests that prolonged administration of low dose capsaicin, which we may be consumed by humans from eating a spicy meal, may not be enough to stimulate VR1. These results indicate that the vehicle additives Tween 80 and 10% ethanol, which are used to solubilize capsaicin, might activate the receptors and enhance mucosal defence mechanisms.

Epithelial localisation of VR1 has been previously shown in humans in the urothelium of both the urethra and bladder (26). Faussone-Pellegrini (8) reported that VR1 labelling was found in the parietal
cells, in the form of intracytoplasmic granules, of the human stomach. Olah et al. (33) reported a TRPV1 location in the endoplasmic reticulum of DRG neurons. As Lazzeri et al. (26) reported that VR1 might also be localised in the endoplasmic reticulum of epithelial cells.

In the present study, the medium immunoreaction to the VR1 was observed in the Brunner’s glands both in the vehicle treatment and in the experimental groups, whereas VR1 immunoreactivity was weak in the control group. On the other hand, Moore et al. (30) demonstrated that Brunner’s glands are innervated by cholinergic vagal fibres, but not by capsaicin-sensitive or intrinsic enteric nerves, in this way capsaicin dilated arterioles had no effect on Brunner’s glands. In addition, Kaunitz et al. (23) demonstrated that the secretion of Brunner’s glands played a role in the duodenal defence mechanism. The detection of VR1 immunoreaction in the Brunner’s glands, as it was expressed by Kaunitz et al. (23) may lead to the idea that the glands play a role in mucosal defence.

We also observed that VR1 immunoreactivity was expressed in the longitudinal muscle layer and myenteric plexus. VR1 immunoreactivity was particularly more intensive in the myenteric plexus. Some of the intrinsic enteric nerves in the myenteric plexi, particularly in the guinea pig ileum and colon seem to express VR1 (3). Furthermore, vanilloid receptors have also been demonstrated in porcine-cultured myenteric neurons (25). Our immunohistochemical findings of VR1 localisation are in agreement with the previous reports on the distribution of VR1 throughout the myenteric plexus and the external muscle layers (3, 31). Previous investigators have shown that lower doses of capsaicin affected gastric motility (21) and gastric blood flow (1) in rats. These results were consistent with the data showing that capsaicin elicited contraction and relaxation of smooth muscle in the rat duodenum (15). VR1 receptors suggest that the capsaicin receptor expressed by neurons in the myenteric plexi, might also contribute to the development of enhanced intestinal motility and secretion. The results indicate that in the experimental group, low dose and prolonged administrated of capsaicin did not produce degeneration of the capsaicin sensitive nerve endings.

The best established effect of capsaicin on the gastrointestinal tract is vasodilatation. VR1 plays an important role in the control of localised blood flow, with VR1 in a key location to control the local release of vasoactive neurotransmitters (16). The major transmitter is CGRP. The CGRP containingafferent nerve fibres have also been reported around blood vessels in gastric mucosa (40). In many pharmacological studies it is suggested that capsaicin inhibits aggravation of gastric lesions, probably by increasing the gastric mucosal blood flow via release of nitric oxide and sensory neuropeptides such as CGRP (13). In the present study, VR1 expression was identified in the endothelial cells of rats except for the control group; hence, VR1 may play an important role in the control of mucosal blood flow and result in mucosal protection.

These results indicate that prolonged administration of a low dose of capsaicin, which may be also consumed by humans during eating a spicy meal, may not be sufficient to stimulate VR1. Additionally, the vehicle additives, Tween 80 and 10% ethanol, which are used to solubilize capsaicin, may activate the protection mechanism in the mucosa epithelium and also stimulate the capsaicin sensitive afferent neurons by VR1 to increase mucus secretion.

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References

13. Holzer P., Lippe I.T.: Stimulation of afferent nerve ending by intragastric capsaicin protects against ethanol-


