has been known for a long time that this drug stimulates nociceptive nerve endings ("receptors" in the physiological sense), thus causing both afferent discharge and release of biologically active substances from these nerve endings. The receptive protein on these endings is the TRPV1 capsaicin receptor (in the pharmacological sense), a cation channel-linked receptor activated by noxious heat, low pH, and chemicals such as capsaicin1,2. The primary role of the TRPV1 is most probably to signal noxious heat to the sensory nerve endings whereupon it is located. Thus, its chemical sensitivity (towards capsaicin from Capsicum annuum or resiniferatoxin from Euphorbia resinifera) may be just an accident of nature. In fact, the mode of action of capsaicin is to lower the nociceptive heat threshold to such an extent that core body temperature or even room temperature can activate the receptor molecule. It should be noted that activation also follows an exposure of the receptor molecule to protons, i.e., low pH. Opening of the non-selective cation channel encountered leads to an influx of Na+ and Ca2+ (also some efflux of K+); as end effect, the membrane of the nerve ending will become depolarized and gives rise to afferent signals1,3-6.

The work of Jancso and coworkers has demonstrated that activation of capsaicin-sensitive afferent nerve endings is associated with plasma extravasation in the skin and mucous membranes7,8. This effect has been attributed to biologically active substances, released from these nerve endings upon activation. It has also been noted that such a "local efferent" function of afferent neurons is dependent on Ca2+ that enters through the TRPV1 ion channel, hence, capsaicin can lead to release of biologically active substances in a way that does not involve tetrodotoxin-sensitive, voltage-dependent Na+ channels.
Studying the effects of an activation of capsaicin-sensitive nerves in visceral smooth muscles can serve different purposes. (1) It should be demonstrated that the particular organ under investigation possesses capsaicin-sensitive afferents whose activation leads to “local efferent” responses in the viscus. (2) It is also of interest what the physiological or pathophysiological roles of the “local efferent” responses may be. (3) If Dale’s principle is applicable to afferents, the same chemical mediators are released in both central and peripheral endings of capsaicin-sensitive neurons. This may allow an identification of sensory transmitters and modulators in more simple experimental arrangements (e.g., in an organ bath experiment) than studying the spinal dorsal horn. When assessing possible mediating roles of different transmitters in smooth muscle effects of capsaicin, priority has been given to pharmacological evidence (first of all, the judicious use of specific neurotransmitter antagonists) provided by the researchers.

Excitatory Effects of Capsaicin on Gastrointestinal Preparations and the Mediators Involved

Szolcsányi and Bartho were the first to systematically study the intestinal effect of capsaicin and of the antidromic stimulation of capsaicin-sensitive nerves to demonstrate this new type of nerve-mediated motor response in the guinea-pig small intestine and taenia caeci, as well as in the rabbit small intestine and guinea-pig duodenum.

It has been found that, in the longitudinal muscle of the guinea-pig ileum and taenia caeci, capsaicin causes a predominantly cholinergic contraction which, however, is totally prevented by a previous degenerative section of the mesenteric nerves. The response is reproduced by mesenteric nerve stimulation (following sympathetic blockade). The excitatory response evoked in this way is strongly inhibited by capsaicin pretreatment. A cholinergic involvement in this response was surprising, since capsaicin-sensitive afferents were not expected to contain and release acetylcholine. We assumed that capsaicin-sensitive sensory nerves released unknown mediator(s) that in turn excite cholinergic neurons of the myenteric plexus, and the latter evoke cholinergic contraction of the longitudinal smooth muscle. At that stage, we could not demonstrate a mediating role of even substance P in the response, most probably because we used a substance P receptor desensitization procedure that was only effective against the direct smooth muscle-contracting effect of this peptide. In the guinea-pig duodenum or in the small intestine of (young) rabbits mesenteric, perivascular nerves seem to contain functional cholinergic preganglionic and capsaicin-sensitive fibres. Thus, the entire contractile response to mesenteric nerve stimulation is abolished by in vitro capsaicin desensitization plus a ganglion blocker. Since it has been published that substance P may have an acetylcholine-releasing effect on the guinea-pig ileum and this action shows less tachyphylaxis than the smooth muscle contracting one, the two teams jointly investigated the action of a more powerful substance P desensitization procedure on the excitatory action of capsaicin and of mesenteric nerve stimulation in the guinea-pig ileum. We found that a massive tachyphylaxis to substance P diminishes the effect of both capsaicin and mesenteric perivascular nerve stimulation. It was concluded that a substance P-like peptide participates in the activation of cholinergic motoneurons in the course of sensory stimulation. A similar conclusion as to the effect of capsaicin has been reached in a parallel study by Chahl, who also confirmed the acetylcholine-releasing effect of substance P in the guinea-pig ileum (Figure 2).

Later studies by other teams confirmed these findings in both the small and the large intestines of the guinea-pig. An acetylcholine-releasing effect of capsaicin has been detected via a direct measurement of H-acetylcholine release.

While periarterial stimulation failed to reveal the presence of any parasympathetic preganglionic nerves in the ileum or caecum of the guinea-pig (see above) we found pharmacological evidence for a presence of a capsaicin-resistant, most probably cholecystokininergic contractile effect in response to mesenteric nerve stimulation. This response was found to be sensitive to lorglumide, an antagonist acting on cholecystokinin receptors. It has been tentatively sug-
suggested that the cholecystokinin-containing neurons encountered are “centrifugal” myenteric neurons with cell bodies in the myenteric plexus but the axons, at least part of them, running in the mesenteric perivascular nerves to the celiac ganglion.

If tachykinins play a role in the excitatory effect of capsaicin in the guinea-pig ileum (as activators of myenteric cholinergic motoneurons), the most probable receptor type encountered should be the tachykinin NK₁ receptor, whose activation results in a completely neurogenic response (hence is similar to the response to capsaicin). Yet, a combination of tachykinin NK₁ and NK₃ receptor antagonists was necessary to partially inhibit the contractile response or [³H]-acetylcholine release to capsaicin, while an inhibition of either of these receptor types alone failed to cause a significant reduction. An NK₂ antagonist was ineffective alone or in any combination. These findings are consistent with the inhibitory effect of a non-selective tachykinin receptor antagonist on the capsaicin-induced contraction in the guinea-pig small intestine.

Capsaicin causes a facilitation of the peristaltic reflex evoked by an elevation of the intraluminal pressure (i.e., the drug lowers the pressure threshold for evoking reflex emptying of the intestine). The neurotransmitter background of this effect has not been analyzed.

Several putative neurotransmitters have been examined for a possible participation in the excitatory effect of capsaicin in the guinea-pig small intestine. Their inhibitors were tested either alone or in combination with tachykinin NK₁ + NK₃ receptor antagonists. It has been found that the purinoreceptor antagonist PPADS inhibits the capsaicin-evoked excitatory response if administered together with tachykinin antagonists, but fails to do so if given alone. Thus, here again, there seems to be a “supra-additive” relationship between the two mechanisms (see Figure 2). Interventions that apparently fail to affect the response of the ileum to capsaicin, including receptor antagonisms of VIP, cholecystokinin, GABA or endothelins, tachyphylaxis to corticotrophin releasing factor (CRF), NO synthesis inhibition, tachyphylaxis to the neuronal stimulatory action

Figure 2. Motor effects of capsaicin-sensitive afferent neurons in the small intestine of the guinea-pig. Pathways not yet fully elucidated are drawn with dotted lines (for details see text). Abbreviations: SP, substance P; NKA, neurokinin A; NK₁, NK₂, NK₃, tachykinin NK₁, NK₂, NK₃ receptors; ACh, acetylcholine; M, muscarinic receptor; P₂, P₂ purinoceptor; CGRP, calcitonin gene-related peptide-1 receptor; + symbols, excitatory, - symbols, inhibitory influences.
of CGRP or muscimol (a GABA_A receptor agonist) have been recently reviewed (2).

The contractile effect of capsaicin on the guinea-pig common bile duct also seems to involve an interplay of tachykinin receptors, the most effective against capsaicin being a combination of tachykinin NK_1 + NK_2 + NK_3 receptor antagonists. The guinea-pig gallbladder also contracts in response to capsaicin. The electrophysiological correlate of the excitatory effect of capsaicin on the sphincter of Oddi and gall bladder seems to be a slow depolarization (caused by exogenous or endogenous tachykinins) in myenteric neurons, largely mediated by NK_1 receptors (24,37).

Other gastrointestinal preparations that show capsaicin-sensitive excitatory responses include the rat (31) and cat stomach (32), rabbit colon (33), guinea-pig distal colon (34), oesophagus (22,28,35), and mouse small intestine (36). Tachykinins are usually involved in these responses, and in some of the preparations a participation of cholinergic neurons can also be verified (2). Interestingly in dogs, contractions of the stomach in response to intragastric capsaicin are inhibited not only by atropine or a tachykinin NK_1 receptor antagonist, but also by the 5-HT_3 receptor blocker ondansetron (37).

Capsaicin, administered to the distal oesophagus evokes contraction of the lower oesophageal sphincter in the dog in vivo, probably as a result of local reflex activation (38). Neurochemical studies have demonstrated a release of substance P, neurokinin A- and CGRP-like immunoreactivity from the stomach, upon exposure to capsaicin (24, 42). Immunohistochemical studies reveal abundant tachykinin-like immunoreactivity in the gastrointestinal tract, but only a small fraction of this is associated with capsaicin-sensitive neurons; the majority is present in intrinsic nerves (17,43). In fact, intrinsic tachykinergic neurons may participate in the excitatory effect of capsaicin in the guinea-pig ileum, though their role seems much smaller than that of acetylcholine. It is likely that tachykinins, originating from capsaicin-sensitive neurons release some tachykinins from intrinsic neurons (2).

In the pig antrum, release of PACAP(1-38) with subsequent stimulation of cholinergic and tachykinergic structures seems to be involved in the capsaicin-induced stimulation of motility (44). An interesting feature of the capsaicin-induced excitatory response, at least in the mouse distal colon (longitudinal muscle) is that it is partly sensitive to desensitization to allyl isothiocyanate, a stimulant of the TRPA1 channel, and vice versa. This is taken as evidence for extrinsic sensory nerve endings harboring both TRPV1 and TRPA1 (45). Since the contractile effect of allyl isothiocyanate is sensitive to tetrodotoxin and atropine, the response to this substance shows some similarities to the effect of capsaicin in the guinea-pig ileum. A pharmacological analysis of the capsaicin-evoked response has not been performed in this study.

Some drugs other than capsaicin seem to excite the gut via TRPV1 or at least through capsaicin-sensitive nerves. The endocannabinoid anandamide, besides inhibiting evoked acetylcholine release (an effect mediated by cannabinoid CB_1 receptors) enhances basal acetylcholine release, apparently via TRPV1 stimulation (46,47). The study of Mang et al. (46) confirmed that the excitation of cholinergic neurons by sensory nerve endings is probably mediated by tachykinins, acting at NK_1 plus NK_2 receptors. In preparations of the gastrointestinal tissue, excitatory reactions to protease-activated receptor (PAR) stimulating peptides have also been found to be sensitive to capsaicin pretreatment (48,49). It is, however, not clear if an inhibition of the contractile responses by capsaicin pretreatment reflects a participation of extrinsic sensory neurons or is a consequence of a non-specific smooth muscle relaxant effect of this drug (see below), since capsaicin, in a low spasmyloytic concentration (10 µM) was present in the organ bath in the point of time of PAR stimulating peptide administration. Hence, this potentially intriguing point probably needs to be re-examined.

Inhibitory Effects [Specific and/or Non-Specific] of Capsaicin on Gastrointestinal Preparations Taken from Animals

Since capsaicin-sensitive afferents have been shown to contain CGRP (3) and CGRP relaxes the guinea-pig ileum (38), we tested the effect of capsaicin on atropine-treated, precontracted longitudinal preparations of the guinea-pig. A moderate relaxant effect has been detected that was sensitive to capsaicin pretreatment, mesenteric denervation and CGRP tachyphylaxis (51), but not tetrodotoxin, indicating that myenteric neurons are not involved. Later on, a CGRP antiserum (54), CGRP tachyphylaxis (52) and the CGRP antagonist hCGRP(8-37) has also been found to reverse or prevent the inhibitory effects of capsaicin on
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both the longitudinal and circular muscles of the guinea-pig ileum. Thus, it is conceivable that a CGRP-like substance is released from sensory neurons and it inhibits the smooth muscle directly.

Following initial stimulation (see above), capsaicin causes a specific inhibitory action on the peristaltic reflex in the guinea-pig ileum. This effect is partially inhibited by the CGRP antagonist hCGRP(8-37) or an inhibitor of NO synthesis. We feel that this favours the involvement of endogenous CGRP in this effect more than that of NO, since NO synthesis inhibition, unlike the CGRP antagonist, stimulated the reflex at basal conditions as well, thus suggesting a modulating role of endogenous NO.

Another inhibitory effect of capsaicin (in specific concentrations around 1 µM) on the guinea-pig ileum is on the cholinergic nerves, as detected via measuring [H]-acetylcholine release from the field-stimulated myenteric neurons. As pointed out by these authors, this may represent a direct action on the cholinergic motoneurons and unrelated to TRPV1 (see below). On the other hand, the TRPV1-mediated acetylcholine-releasing effect of capsaicin is enhanced by an inhibition of NO synthase, indicating that neuronal activation in the course of the specific capsaicin action also leads to NO release that partly blunts the excitatory response (see Figure 2).

While describing the specific, probably sensory neuron-mediated inhibitory effect of capsaicin in the guinea-pig small intestine, we stressed (again) the existence of a non-specific smooth muscle-relaxant property of this drug, appearing in concentrations above some 1-3 µM in vitro. This action is reversible upon rinsing and allowing the tissue to recover, i.e., it does not undergo desensitization. The peristaltic reflex of the guinea-pig ileum is also repeatedly and reversibly inhibited by a high concentration of capsaicin. Unfortunately, this non-specific effect of capsaicin and similar substances (including capsazepine) is frequently disregarded. Capsaicin is often used as “sensory nerve blockceptor “tachykinin depletor” and capsazepine as TRPV1 antagonist without the necessary precautions; this may lead to many false positive results concerning the participation of capsaicin-sensitivenerves in some intestinal excitatory responses.

Several groups have analyzed the possible mechanism of the spasmolytic effect of capsaicin and concluded that it is based on Ca\(^{2+}\) channel inhibition. In addition to this, non-L-type Ca\(^{2+}\) channels, Ca\(^{2+}\)-release from intracellular stores and the contractile machinery within the smooth muscle cells may also be inhibited, while a stimulation of 4-aminopyridine-sensitive K\(^{+}\) channels may be activated.

Both specific and non-specific inhibitory effects of capsaicin in the guinea-pig distal colon longitudinal muscle in vitro have been described by Maggi and coworkers. Interestingly, the specific inhibitory action was sensitive to tetrodotoxin, a finding that is similar to the inhibitory effect of capsaicin on the rat duodenal longitudinal or the mouse distal colon circular muscle and may be explained by an activation (possibly by sensory nerve endings) of intrinsic inhibitory nerves or by an axon reflex arrangement (i.e., release of sensory transmitters partly from antidromically-activated capsaicin-sensitive sensory nerve endings). The specific relaxant response of the guinea-pig proximal colon circular muscle to capsaicin is not influenced by an inhibition of NO synthesis and might be mediated by CGRP.

Other gastrointestinal preparations that show inhibitory responses to capsaicin include the rat and rabbit colon, the guinea-pig biliary system, the oesophageal muscle of the mouse, rat and hamster, the rat gastric fundus and the ferret lower oesophageal sphincter. Evidence has been presented that capsaicin inhibits vagally-induced twitch contractions by releasing tachykinins that in turn liberate NO from intrinsic neurons.

There is evidence that capsaicin-sensitive neurons mediate the distension-evoked relaxation, but not that evoked by vagal stimulation, in the guinea-pig stomach in vitro. In anaesthetized rats, distension-induced gastric motility is suppressed by capsaicin, due to the release of endogenous CGRP. In the rat gastric corpus, capsaicin causes an apparently specific inhibitory effect; neither VIP, nor CGRP seems to participate in this response. In the rat gastric fundus, capsaicin induces concentration-dependent relaxations at concentrations (0.1-10 µM) at which this compound elicits mainly specific effects. Capsaicin-induced relaxations are reduced by \(\alpha\)-chymotrypsin, but not by desensitzation to CGRP or a CGRP antiserum, and by L-NOARG, a NO-synthase inhibitor. These findings suggest that NO and a peptide different from CGRP are involved in capsaicin-evoked relaxation of the rat gastric fundus.
NO-Mediated Inhibitory Effects of Capsaicin on Human Gastrointestinal Preparations

The team of C.A. Maggi described a marked relaxant effect of capsaicin in the human small and large intestine. In fact, it was difficult to demonstrate any excitatory action of this drug on human intestinal preparations. Evidence has been presented against a mediating role of CGRP in this response. VIP seemed more likely to participate. Tetrodotoxin failed to inhibit the response to capsaicin, thus rendering a participation of enteric intrinsic neurons unlikely.

As late as the beginning of the present decade the neurotransmitter background of the relaxant effect of capsaicin in the human intestinal circular muscles was re-examined by our team; we found that in the ileum, appendix, proximal and sigmoid colon an inhibition of NO synthesis strongly suppresses the response to capsaicin. We fully confirmed the insensitivity of this effect to tetrodotoxin. Experiments are still in progress to find out more about the mediator background of this effect; e.g., a possible participation of interstitial cells of Cajal is also considered. The most simple explanation, however, would be that NO originates from the sensory neurons themselves. There are morphological data concerning the presence of NO synthase-like immunoreactivity in dorsal root ganglia.

In vivo Effects of Capsaicin-Sensitive Neurons on Gastrointestinal Motility in Health and Disease

Animal studies indicate that intact capsaicin-sensitive nerves are no prerequisite for a normal propulsive motility of the gastrointestinal tract. This probably reflects the fact, pointed out in both early and more recent studies, that intrinsic intestinal neurons are not influenced by capsaicin desensitization, i.e. rendering the capsaicin-sensitive neurons unresponsive to any kind of stimulation, though there may be differences between the capsaicin-sensitivities of these neurons’ "release" (i.e., "local effector") and afferent functions. In the rat, immunoreactive substance P contents of the intestinal tract is not changed by capsaicin desensitization. At the level of the oesophagus or stomach, however, motility may be influenced by capsaicin-sensitive neurons. In dogs in vivo, capsaicin applied in the distal oesophageal reflexes lower oesophageal sphincter contraction, probably by activation of a local reflex mechanism that may involve tachykinin receptors. Intragastric capsaicin does not influence gastric emptying in anaesthetized rats. Extrinsic sensory neurons may be involved in the effects seen with intestinal anaphylaxis. A capsaicin-sensitive disruption of the normal motor pattern of the proximal small intestine of albumin-sensitized rats upon antigen challenge has been reported. In these latter experiments, the sites of recording of myoelectric complexes and the administration of albumin were close to each other, distant effects (reflex or hormonal-like) were therefore not detected.

Capsaicin has been found ineffective on human lower oesophageal sphincter strips in vitro. In vivo studies with intra-oesophageal capsaicin-containing solutions indicated a stimulatory effect on oesophageal motility and contraction of the lower oesophageal sphincter in healthy volunteers. Gastric emptying is prolonged but this is compensated for by an increased small intestinal propulsion; as an end effect, orocecal transit time is not altered. This fits well with reports on a relaxation of the stomach in man, the in the rat in vivo, in response to capsaicin. On the other hand, an increase in orocecal transit time has also been reported.

The afferent function of capsaicin-sensitive neurons seems to be involved in reflex inhibition of gastrointestinal motility in response to peritoneal stimuli in rats, the efferent limb being the sympathetic nerves. A similar effect has been reported to be mediated by CGRP. Capsaicin-sensitive afferents are also likely to mediate the inhibition of gastrointestinal motility in rats in response to i.p. endotoxin, p.o. ethanol, intraduodenal glucose, intraileal acid, lipids, or capsaicin, ischaemia-reperfusion, or colitis. Inhibition of gastric emptying by intragastric peptone, acid, or hyperosmolal saline is dependent, at least in part, on an intact capsaicin-sensitive innervation. Some capsaicin-sensitive gastrointestinal effects may be mediated by a local effect, a short-loop reflex or a reflex involving the central nervous system. Intragastric capsaicin activates both vagal and spinal afferents, as indicated by an enhancement of c-fos expression in the nucleus tractus solitarii and in lamina 1 of the dorsal horn in the distal thoracic spinal cord.
ported by Yiangou et al.\textsuperscript{102} and Chan et al.\textsuperscript{103}. The role of capsaicin-sensitive nerves in functional gut disorders is dealt with in recent reviews\textsuperscript{4,6}.

**Conclusions**

There is no doubt any more about the existence of capsaicin-sensitive sensory innervation of the gastrointestinal tract. Not only subserve these nerves afferent functions (participating in visceral sensations and reflexes) but also influence gastrointestinal functions, such as motility, by releasing biologically active substances. The “local efferent” roles of capsaicin-sensitive nerves and the mediators thereof seem to show large interspecies variation. Tachykinins are excitatory and CGRP relaxant neuroeffector transmitter of these nerves in some animal species, whereas NO largely mediates the inhibitory effects of capsaicin in the circular musculature of the whole length of the human intestinal tract.

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