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Review

# Neuroprotection with the red pepper agent capsaicin

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## ABSTRACT

Capsaicin has a long history of anecdotal benefit for counteracting pain, heat and itch, and a majority of research into its clinical application has focussed on its analgesic potential. However, capsaicin elicits several distinct cellular activities in addition to analgesia. Hence its therapeutic potential is being actively explored in a wide range of acute and chronic pathologies including stroke and neurodegeneration, inflammatory disease and sepsis, gastrointestinal disorders and cancer. Capsaicin generates cellular responses via its physiological receptor, the transient receptor potential cation channel subfamily V member 1 (TRPV1). TRPV1 are polymodal receptors present in both peripheral nerves and the central nervous system. They primarily function as sensors of noxious stimuli, such as heat, acid and pro-inflammatory molecules, and may play an important role in body temperature regulation. Activation of TRPV1 by capsaicin can sensitise sensory neurones or modulate nerve sensation and function to produce analgesia. Interestingly, capsaicin can also exert paradoxical actions on inflammatory processes, cell viability and vascular tone. These cellular activities are largely mediated via agonism of TRPV1 that elicits multiple effects including receptor activation to desensitisation and dysfunction through $Ca^{2+}$-dependent processes. Capsaicin's ability to reduce neuron excitation and activation is thought to be the basis of its therapeutic benefit in pain perception and other forms of neuroprotection associated with TRPV1. However, capsaicin has several cellular effects that show benefit including anti-inflammation, anti-oxidation and anti-proliferation and some of these actions are independent of neurones and TRPV1. Hence, capsaicin has considerable therapeutic development potential in inflammation, neurotoxicity and tissue degeneration and may be beneficial in neuroprotection and in pathologies associated with the cardiovascular and central nervous systems.

**KEYWORDS:** capsaicin, neuroprotection, stroke, Alzheimer's, Parkinson's

## 1. Introduction

Capsaicin (trans-8-methyl-*N*-vanillyl-6-nonenamide; chemical structure shown below, Figure 1) is a member of the vanilloid group of chemicals. Capsaicin is the primary capsaicinoid produced naturally by vegetable peppers of the *Capsicum* genus. Capsaicin and related compounds are responsible for the piquancy of chilli that manifests as a burning or heat sensation in the oral cavity when ingested. It is used globally as an important food and flavour enhancer. However capsicum extracts have been noted earlier as having significant physiological effects including a burning sensation on the skin, hyperaemia, capacity to decrease blood pressure and increased salivary, gastric and intestinal activity. The most notable effect of applied capsaicin is the phenomenon of pain alleviation, also referred to as counter-irritation [1].

Chemically purified capsaicin is a volatile, colourless, odourless, crystalline compound, which readily dissolves in lipid and alcohol due to the presence of a hydrophobic hydrocarbon tail (Figure 1).

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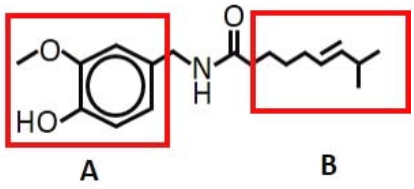


**Figure 1.** Chemical structure of the red pepper extract capsaicin (trans-8-methyl-*N*-vanillyl-6-nonenamide). The phenyl ring (A) and octyl side chain (B) impart significant hydrophobicity to the molecule, which facilitates passive diffusion through membrane bilayers. Thus, the lipophilic nature of capsaicin makes it largely insoluble in water, but freely soluble in alcohols and edible oils.

Its relatively high lipophilicity allows it to readily cross lipid bilayers, and passively diffuse into biological membranes [2]. Naturally occurring capsaicin is found as the *trans* structural isomer with the presence of the -$CH(CH_3)_2$ moiety and the long hydrocarbon (octyl) chain on adjacent sides of the double bond. Steric hindrance in the *cis* form is less energetically favoured [2]. The capsaicin molecule also contains an aromatic ring and amide bond similar to other vanilloid compounds. Attached to the phenyl ring is a hydroxyl (-OH) and methoxyl (-$OCH_3$) group, both of which are essential to the interaction of capsaicin with its physiological receptor, transient receptor potential vanilloid 1 (TRPV1), through which most of the biological activity of capsaicin is thought to be mediated [3].

Activation of TRPV1 channels in sensory neurones by capsaicin initially leads to activation of pain pathways. However, continued activation of TRPV1 by capsaicin can inactivate and desensitise neurones to further stimuli. This well-known desensitising ability of capsaicin has been therapeutically exploited and capsaicin is widely used to treat chronic neuropathic pain and itch [1]. However, research on the thermogenic and neurogenic effects of capsaicin has recently focussed more attention on its therapeutic potential in a diverse range of pathologies. In particular, capsaicin has been found to exert neuroprotection in models of acute cerebral ischaemia and chronic neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. The aim of this article is to summarise the pharmacology of capsaicin and its current therapeutic use and highlight its potential role in neuroprotection.

## 2. Pharmacokinetics

### 2.1. Absorption and distribution

Capsaicin pharmacokinetics and distribution have been extensively studied due to its potential therapeutic value in pain management. There are several possible routes of administration including topical, oral, intragastric, intravenous, intraperitoneal and subcutaneous. The route of administration determines the tissue distribution of capsaicin. Capsaicin exhibits a relatively long half-life (~24 h) *in vivo* following topical administration due to a combination of rapid absorption and slow biotransformation [4, 5]. The maximal concentration is dependent on its relative solubility in vehicle; capsaicin readily dissolves in alcohol and hence higher concentrations can be achieved using alcohol-based solvents [4]. These properties together with the fact that capsaicin is tolerated well in the diet (suggesting generalised low toxicity) led to the development of a wide range of capsaicin-based creams, lotions and patches for use in pain management [6].

Tissue distribution following oral or intragastric administration has been studied extensively in rats. Capsaicin is rapidly absorbed from the gastrointestinal tract, with approximately 85% absorbed within the first 3 h [7, 8]. Donnerer and co-workers have shown that high doses of capsaicin delivered via the intragastric route can lead to saturation of the absorption process [9]. A comprehensive study by Suresh and Srinivasan [8] detailed changes in tissue distribution over eight days following a single oral dose of capsaicin. After 1 h, blood and intestine contained the highest concentration of capsaicin, indicating rapid absorption, followed by the liver at 3 and the kidney after 6 h. By 96-h post-supplementation the capsaicin levels dropped below detectable levels [8]. The majority of administered capsaicin was absorbed (~94%) and a minority was excreted in faeces and urine (~6.3%); peak excretion occurred during the first day [8]. Orally administered capsaicin had a shorter half-life (~8 h) compared to topically administered capsaicin (~24 h), possibly due to effective hepatic metabolism by cytochrome $P_{450}$ enzymes [4, 5, 8].

Intravenous, intraperitoneal and subcutaneous injections have been typically used to deliver capsaicin in experimental animal models. Intravenous injections facilitate rapid entry of capsaicin into the central

nervous system such that 3 min post-intravenous injection, the brain and spinal cord contained 5-fold higher concentrations compared to blood [10]. However, accumulation of capsaicin within the central nervous system (CNS) is toxic which explains the lower $LD_{50}$ value (0.6 mg/kg) compared to subcutaneous (9.0 mg/kg) or intraperitoneal (6.5 mg/kg female rats; 7.7 mg/kg male rats) modes of administration in rodents [11]. Capsaicin administered subcutaneously was detected early and levels continued to increase over 24 h reflecting its passage through tissue before reaching the systemic circulation [10]. In contrast to oral and topical administration, the half-life of intravenous and intraperitoneal injections is quite short, possibly due to rapid entry into the liver, the main site for metabolism [4, 5, 8, 12]. Studies by Saria *et al.* (1982) also showed that capsaicin concentrations in the CNS, blood and adipose tissue were similar following subcutaneous injections, thus suggesting that the hydrophobic nature of capsaicin enabled molecules to easily pass the blood-brain barrier [10]. This characteristic makes capsaicin a key candidate in therapies targeting cerebral tissues.

### 2.2. Metabolism and excretion

Metabolism of capsaicin in humans, rats and mice produces up to five primary metabolites. Reilly *et al.* (2003) and Chanda *et al.* (2008) studied capsaicin metabolism using lung and liver microsomes, liver S9 fractions, and skin from a variety of species, including humans [5, 13]. These independent research groups identified three major metabolites 16-hydroxycapsaicin, 17-hydroxycapsaicin and 16,17-dehydrocapsaicin that were assigned and characterised using liquid chromatography coupled with mass spectrometry, and two other minor metabolites vanillylamine and vanillin (the predominant metabolites produced by human skin) [5, 13, 14]. Interestingly, Chanda and co-workers observed that the rate of biotransformation was greater in microsomes than S9 fractions, and that rates of metabolism could be saturated; less extensive metabolism was observed at 10 µM capsaicin compared to the lower 1 µM dose [5]. Capsaicin is predominantly metabolised by the liver and involves the microsomal enzyme cytochrome $P_{450}$ [13, 14].

Microsomal preparations derived from other tissues such as the brain, spinal cord, skin, lung and intestines are also capable of metabolising capsaicin, albeit at a slower rate than the liver [5, 9], which likely reflects the relative content of cytochrome $P_{450}$. Metabolites derived from capsaicin generally show enhanced hydrophilicity and consequently the majority of capsaicin metabolites are excreted in urine [14]. The majority of orally administered capsaicin is absorbed; the remainder is excreted unchanged in faeces with a very small portion in urine [8, 14].

There are very few studies on the activity of capsaicin metabolites, although this is particularly important when considering potential therapeutic use. Researchers Surh and co-workers [15] observed that aliphatic hydroxylation of capsaicin produced the more polar compound 17-hydroxycapsaicin (structure shown in Figure 2) that may affect its ability to cross the blood-brain barrier. They also showed that both 17-hydroxycapsaicin and vanillylamine lacked the pungency and anti-nociceptive activity of parental capsaicin [15], possibly due to limited agonism of the TRPV1 receptor.

A number of chemically modified derivatives of the compounds 16,17-dehydrocapsaicin and vanillyamine have been developed and patented (sourced from PubChem – open chemistry database https://pubchem.ncbi.nlm.nih.gov/). For example, N-octyl-N'-vanillyl urea is known to modulate flavour (US PCT /2010129515) and is listed as a stimulator of $Ca^{2+}$-ion uptake in rat spinal sensory neurones. Other derivatives of 16,17-dehydrocapsaicin are also listed as flavour modulating agents that also exhibit anti-inflammatory activities.

## 3. Mechanism of action

Noxious chemical, mechanical or thermal stimuli stimulate pain perception through sensory neurones or nociceptors. Sensory neurones are present in many peripheral tissues and transmit pain signals to the central nervous system. When activated, they not only indicate pain/injury, but also release bioactive molecules such as neuropeptides with direct localised effects on tissues or indirect systemic effects via links to the central nervous system. Sensory neurones appear to participate in both physiological and pathophysiological processes including development, homeostasis, inflammation and disease [16].

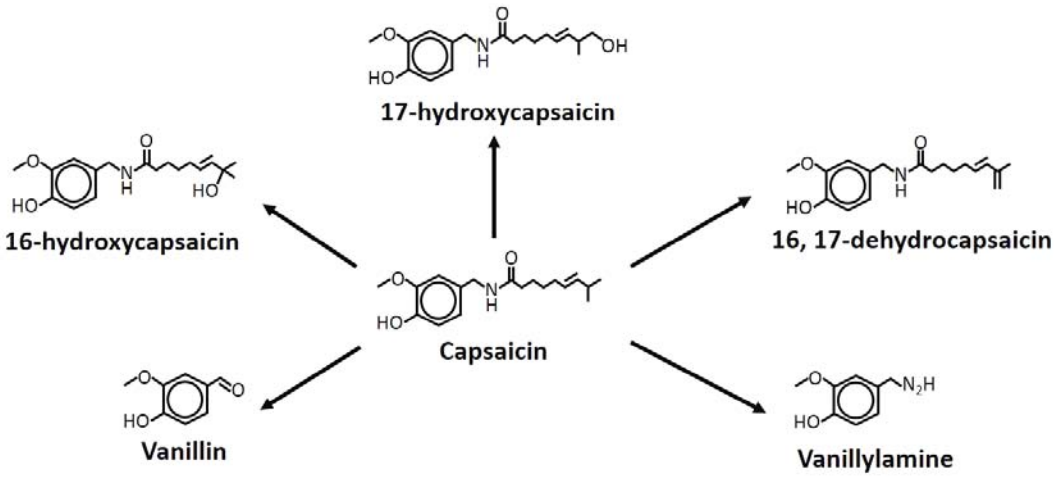


**Figure 2.** Chemical structures of the five most commonly reported metabolites of capsaicin. Changes to and/or removal of the hydrophobic tail affect the biological activity and ability to cross the blood-brain barrier. The metabolites 17-hydroxycapsaicin and vanillylamine show increased partitioning into the aqueous phase suggesting a decreased ability to cross lipid membranes [15]. The biological activities of other potential metabolites shown are not clear; however, chemically modified derivatives of these metabolites appear in the patent literature with varying biological activities.

The nociceptor properties of sensory neurones were predominantly characterised through their sensitivity to and activation by capsaicin [17]. However, it was known for some time that high concentration or repeated exposure to capsaicin generated a time period whereby the nerve terminal was no longer excitable by capsaicin, or other diverse stimuli, resulting in a prolonged and unique analgesic effect. This dual activity of capsaicin that induces apparent paradoxical effects on sensory nerves has been extensively studied and is the basis for its therapeutic potential in pain management.

### 3.1. Neuronal cells and agonism of TRPV1

Characterisation of capsaicin's action on sensory neurones directly led to the discovery of its physiological receptor, transient receptor potential vanilloid 1 (TRPV1) [18], a member of the larger family of vallinoid or TRPV receptors. Exposure of nerves to capsaicin translates into sensory perceptions of burn, sting or itch and this pain perception is inhibited by TRPV1 receptor knockout *in vivo* [19]. TRPV1 is a transmembrane, non-selective, ligand-operated cationic channel expressed in a number of tissues, predominantly sensory neurones of the peripheral nervous system. TRPV1 has a high permeability for $Ca^{2+}$ ions and capsaicin is a selective, exogenous agonist for TRPV1. The TRPV1 receptor is also activated by noxious heat and pH and by several diverse exogenous and endogenous agonists and thereby integrates multiple sensory inputs. Endogenous agonists include other vanilloids such as anandamide as well as native long-chain unsaturated fatty acids, and oxidised lipids such as leukotrienes and other lipid peroxidation products derived from the activity of lipoxygenase enzymes [16, 20]. Whereas capsaicin may facilitate an action potential in sensory neurones to signal irritant chemical stimuli, endogenous agonists probably induce responses to oxidative stress and noxious pH that arise from inflammation and/or tissue injury.

In neuronal cells, capsaicin induces two distinct cellular responses via TRPV1: activation and attenuation of nociceptor activity. The first involves transient activation of TRPV1 in the sensory neuron, promoting $Ca^{2+}$ influx leading to cell depolarisation and release of vasoactive neuropeptides such as substance P and calcitonin gene-related protein (CGRP) [21]. These neuropeptides induce neurogenic inflammation (vasodilatation, plasma extravasation, leukocyte recruitment) and hypersensitivity, allowing perception of noxious stimuli in the central nervous system. Sensitivity of the TRPV1 receptor can be regulated in several ways. Agonists can sensitise the channel to further stimuli; for example, low pH can potentiate capsaicin-induced inward current [18].

TRPV1 channel excitability can be altered by $Ca^{2+}$-dependent and independent protein kinases (phosphorylation; increased excitability) and phosphatases (dephosphorylation; decreased excitability), while channel activity can also be modulated by calmodulin/$Ca^{2+}$ [22]. In addition to endogenous agonists, inflammatory mediators such as bradykinin and nerve growth factor can sensitise TRPV1 via co-expressed receptors [16]. Further, oxidative stress and reactive oxygen species (ROS) may also act as modulators [23].

High or repeated exposure to capsaicin results in a decrease in TRPV1 activity and an inability of the receptor to respond to further stimuli. The most notable effect of capsaicin on neurones is a large increase in intracellular $Ca^{2+}$ [24] and desensitisation of TRPV1 by capsaicin appears to require $Ca^{2+}$-dependent kinases and signalling pathways involving dephosphorylation, calmodulin and membrane phosphoinositol 4,5-diphosphate [22, 25]. Desensitisation can be followed by a long refractory phase that involves loss of neuron function that is associated with capsaicin's ability to counter pain and irritation. This action on nerves by capsaicin was purported to be due to expenditure of neuropeptide reserves. However, additional mechanisms have been proposed to account for the dysfunction of neurones by capsaicin. For example, capsaicin-induced $Ca^{2+}$ influx is associated with cytoskeletal modifications [26] and inhibition of protein synthesis [27] that can destabilise neurotransmission. Also, large ion influxes can activate $Ca^{2+}$-dependent proteases [28] inducing perturbations in cell signalling and mitochondrial function and osmotic swelling thereby promoting neuron dysfunction and/or neurotoxicity [1]. Further, capsaicin exposure drastically reduces the density of TRPV1 receptors from plasma membranes and cellular pools in a $Ca^{2+}$-dependent manner [29]. Moreover capsaicin can lead to reversible retraction of nerve fibre terminals [30] leading to the loss of sensation.

### 3.2. Non-neuronal cells and cell viability

In addition to its effects on neurones, capsaicin has been shown to be active in non-neuronal cells [31]. Thus, TRPV1 is expressed in various non-neuronal cells including smooth muscle and endothelial cells, leukocytes and keratinocytes. Further, TRPV1 is found in several tissue beds including the central nervous system and in various brain regions including the cortex, hippocampus, dentate gyrus, central amygdala, striatum, substantia nigra, hypothalamus, thalamus and cerebellum [31]. In non-neuronal cells, where capsaicin effects on neurotransmission via TRPV1 are not relevant, the primary documented activity of capsaicin is on cell viability. Indeed expression of cloned TRPV1 in transformed cells leads to necrosis that is $Ca^{2+}$-dependent after exposure to capsaicin [18]. There are many reports of decreased cell viability in cancer cells after treatment with capsaicin [32].

The mechanism(s) whereby capsaicin affects cell viability in cancer cells largely centres on its potential to induce apoptosis via disruption of mitochondrial function, activation of caspase and/or induction of ROS and perturbation of cell signalling pathways [32]. On a cellular level, TRPV1 is found in the cytoplasmic membrane where it controls inward $Ca^{2+}$ currents, in cytoplasmic vesicles where it may regulate $Ca^{2+}$ reserves and in the endoplasmic reticulum where it may regulate $Ca^{2+}$ homeostasis [31]. Capsaicin-induced high intracellular $Ca^{2+}$ through activation of TRPV1 or release from intracellular stores [33] can induce apoptosis by activating proteolytic enzymes or disrupting mitochondria function via altered membrane potential, increased permeability and loss of cytochrome C [34]. Capsaicin can also directly affect mitochondrial function via inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, by competing with ubiquinone [35], thereby dissipating mitochondrial membrane potential.

Whether increased ROS that is associated with capsaicin-induced apoptosis arises from mitochondrial dysfunction is unclear at present as in some studies, capsaicin can induce ROS production that is associated with $Ca^{2+}$, DNA disruption and apoptosis independent of mitochondrial respiration effects [32, 36]. Yet other studies have implicated capsaicin-mediated ROS generation as a central mechanism to stimulate apoptosis in pancreatic cancer cells [37, 38]. For example, capsaicin-induced ROS and mitochondrial membrane potential disruption are associated with apoptosis in pancreatic cancer cells *in vitro* and capsaicin treatment induces apoptosis and suppresses tumour growth *in vivo* [38]. Capsaicin has also been demonstrated to inhibit mitochondrial complex-I and -III activity and ATP

levels in pancreatic cancer but not in normal or oxidative phosphorylation-deficient ($\rho^0$) cells that lack mitochondria [37]. Further, pancreatic tumours in capsaicin-treated mice show evidence of decreased antioxidant enzymes and increased oxidised glutathione, suggesting that oxidative stress is associated with its apoptosis and tumour growth suppression effects *in vivo* [37].

The above positive outcomes in preclinical models of cancer are somewhat tempered by outcomes in human studies that link red pepper consumption with elevated risk of gastric cancer [39]. However, there is a relative paucity of epidemiological data relating chilli pepper consumption to human cancer [32] and all in all capsaicin seems to be well tolerated either in the diet or topically. It remains possible that any cancer-causing effects of capsaicin in humans relates to the presence of carcinogenic contaminants identified in chilli peppers or preparations thereof such as pesticides, aflatoxins and heavy metals, and/or capsaicin metabolites as discussed in detail within [32] and papers cited therein.

The cytotoxic effects of capsaicin in cancer cells, contrast with its relatively benign effects observed in normal or non-malignant cells and also with its high dietary tolerance, may relate to the generally higher metabolic activity observed in cancerous cells. However, recent work suggests these differential effects are due to signalling through diverse cell death pathways. For example, capsaicin increases silent mating type information regulation (SIRT1) deacetylase activity and intracellular $NAD^+$/NADH in foetal lung cells that is associated with autophagy and decreased acetylation of the tumour protein, p53. In contrast, in A549 lung cancer cells, capsaicin was noted to decrease $NAD^+$/NADH and SIRT1 activity in parallel leading to an enhanced acetylation of p53 and increased apoptosis after treatment with capsaicin [40]. Knockdown of SIRT1 inhibits both capsaicin-induced apoptosis and autophagy, further suggesting that capsaicin cytotoxicity involves stimulation of the SIRT1 pathway in cell death pathways [40]. Other natural products reported to activate SIRT1 include the polyphenol resveratrol [41]; however, whether resveratrol activates SIRT1 directly or increases SIRT1 protein expression remains debated [42]. Similarly it is presently unclear whether capsaicin acts to increase SIRT1 protein expression or directly enhances endogenous SIRT1 activity in cancer cells.

### 3.3. Anti-inflammation and anti-oxidation activities

In addition to the above mode of action, capsaicin can affect cell viability via other mechanisms and at high concentrations some of these effects may be TRPV1-independent as capsaicin can passively diffuse cell membranes. For example, capsaicin can inhibit the expression of the pro-inflammatory transcription factor nuclear factor kappa B (NF-κB) [43] that regulates genes involved in cell survival/ proliferation and inflammation. Misregulated or chronically active NF-κB is found in malignant cells that are constitutively active and also in inflammatory diseases. In prostatic cancer cells capsaicin inhibits NF-κB and TNF activation [44]. In other studies, capsaicin has been shown to inhibit growth of tumour cells via reducing superoxide radical anion levels [45]. In this case capsaicin is proposed to modulate ROS-induced activation of NF-κB thereby reducing cancer cell proliferation [45]. In immune cells, capsaicin demonstrates anti-inflammatory action in stimulated macrophages that may be TRPV1-independent and also involving down-regulation of NF-κB [46]. Further, ingestion of capsaicin enhances a discreet population of CD11b and F4/80 positive macrophages in pancreatic lymph nodes, which subsequently expresses anti-inflammatory factors that confer protection against diabetes [47].

Capsaicin can also exert antioxidant functions. For example, it protects against $CCl_4$-induced hepatotoxicity in rats by inducing antioxidant enzyme expression and inhibiting active caspase-3 expression [48]. It has potent free radical scavenging and iron chelating properties [49]. This may be particularly important in the brain, which is susceptible to oxidative damage due to its high oxygen demand and abundance of polyunsaturated lipid in regions of high iron content, and also in Alzheimer's disease where high concentrations of transition metals have been found. Further, these antioxidant actions may also be important in other pathologies involving oxidative stress such as ischaemia/reperfusion injury. Capsaicin can bind to $Fe^{2+}$, potentially mitigating the reactivity of redox active iron in reactions with $H_2O_2$ which form

highly reactive hydroxyl radicals or quinolinic acid, which in turn bind to redox active iron to form quinolinic acid-iron complexes that can induce indiscriminate lipid peroxidation [49]. Further, pre-incubation of macrophages with capsaicin lowers superoxide and nitrite radical generation, and inhibits hydrogen peroxide release both *in vitro* and in rats fed with various dietary lipids and therefore may be effective in lowering inflammation [50].

## 4. Therapeutic use of capsaicin

Capsaicin appears to exert paradoxical cellular actions, obviated via expression of pain stimulation or relief and demonstrated further by differential effects on cell survival and inflammation. There are several reasons for this including that any of the processes facilitated by TRPV1 agonism, such as modulation of $Ca^{2+}$ concentration, activation of kinases or neuropeptide release, can induce pro- or anti-inflammation, vasodilation or vasoconstriction, formation of low levels of ROS for physiological cell signalling and/or apoptosis initiation. Also, TRPV1 is found in a diverse array of cells, both neuronal and non-neuronal and moreover, receptor effects may act intra- and/or extracellularly. In addition, endogenous inflammatory mediators arising from tissue damage or ischaemia can sensitise TRPV1 to other stimuli and affect its activity. Further, capsaicin exerts cellular effects that may be independent or in addition to TRPV1. Hence the cellular actions of capsaicin are likely determined by the underlying (patho)physiology and whether TRPV1 activation (*e.g.*, for anti-inflammation, cancer cell cytotoxicity), or diminution of function (*e.g.*, for pain and excitotoxicity prevention) is central to the developing pathology. Therefore, capsaicin has considerable potential as a natural therapeutic agent in diseases that involve inflammation, hypertension and neurotoxicity.

### 4.1. Current use in pain management

The ability of capsaicin to switch nociceptors from pain sense to pain relief without loss of consciousness is the basis of its therapeutic potential in analgesia. Capsaicin is widely available in a variety of low-to-moderate concentrations as a topical formulation, and early pain intervention studies sought to use capsaicin to desensitise nerve endings. However, repeated topical administration of low dose capsaicin adversely affects mucous membranes, resulting in non-compliance and is not ideal for chronic neuropathic pain. However, a high strength, single-administration 8% dermal patch does show benefit for localised, peripheral neuropathies [6] and was recently approved for non-diabetic neuropathic pain such as postherpetic neuralgia and may also be effective in other neuropathies such as painful HIV neuropathy [51].

Although capsaicin may be effective in neuropathies where denervation or hyperinnervation leads to hyperactive nociception, the high strength capsaicin dermal patch may have only limited effects in large neuropathic zones. However, a recent study shows that topical capsaicin is also effective in traumatic neuropathies and painful diabetic neuropathy that involve large treatment areas [52]. Further, capsaicin can modulate pain associated with rhinitis such as cluster headache [53] and joint pain in osteoarthritis [54]. An analogue of capsaicin, resiniferatoxin, with more potent effects on TRPV1 is currently the subject of clinical trials for use in severe intractable pain as occurring in advanced cancer. Hence, there is continued interest in the benefit of high strength capsaicin, and clinical trials testing for tolerability and epidermal nerve fibre density and sensory function in these pathologies are ongoing [55].

Capsaicin has some pharmacological advantages. It can be applied in high concentrations topically with beneficial effects observed to last for 12 weeks in clinical studies with 3-5 topical applications per day, over a period of 2-6 weeks [56]. Capsaicin has a half-life of ~24 hours [4] as a result of its slow release from the skin reinforcing its long-lasting analgesic effects [57]. Thus its efficacy may be accounted for by the drug's external application, ability to rapidly penetrate the skin and effects exerted close to the application site, in addition to its mechanism(s) of action [58]. It is also widely consumed and well tolerated as a dietary supplement. Further, some of its prominent effects are reversible, for example degeneration of nerve fibres observed with topical capsaicin [30]. Capsaicin is also relatively unique in being able to ablate sensory neuron function and decrease neurogenic inflammation and pain perception. It therefore has potential therapeutic benefit in pathologies in which excitotoxicity may be an underlying cause or a mediator of damage. Capsaicin may also provide

protection in other pathologies via TRPV1 activation in non-neuronal cells or via off-target actions such as anti-oxidation and direct effects on cell signalling pathways leading to anti-inflammation and increased cell survival.

### 4.2. Protective role for capsaicin in the cardiovascular system

TRPV1 activation in sensory nerves is clearly linked to $Ca^{2+}$ influx and neuropeptide release that are normally associated with inflammatory diseases (*e.g.*, rheumatoid arthritis and airway hypersensitivity) and acute pain. This has led to considerable investigation of TRPV1 antagonism in an attempt to block receptor activation and mitigate pain and inflammation. However, several animal studies examining the effects of gene deletion show that TRPV1 activity may actually be required for significant physiological roles in thermoregulation, vascular regulation and neuroprotection, and may be beneficial in pathologies such as ischaemia and hypertension [16].

In the cardiovascular system there is now considerable evidence linking TRPV1 with cardioprotection after ischemic injury. For example, the TRPV1 vasoactive neuropeptide calcitonin gene-related peptide (CGRP) is associated with positive modulation of blood pressure and cardioprotection after ischaemia/ reperfusion injury [59]. Loss of TRPV1 impairs post ischemic heart recovery and promotes infarct expansion and cardiac injury by exacerbating inflammation and abnormal tissue remodelling after myocardial infarction [60, 61]. Further, myocardial ischaemia/reperfusion injury induces TRPV1 expression and eicosanoid formation that protects cardiac neurones [62]. Capsaicin activation of TRPV1 may also be protective in chronic cardiac dysfunction. Thus, chronic dietary capsaicin attenuates high salt diet-induced cardiac dysfunction by improving mitochondrial complex I function in wild type but not TRPV1 knockout mice [63]. Furthermore, resiniferatoxin (an ultrapotent capsaicin analogue) attenuates cardiac fibrosis and apoptosis and improves cardiac compliance in chronic heart failure [64].

Capsaicin has the potential to affect various cellular functions in a wide range of non-neuronal cells by modulation of intracellular $Ca^{2+}$. This may be particularly important in the vasculature as increased $Ca^{2+}$ stimulates endothelial nitric oxide synthase (eNOS). Indeed, dietary capsaicin increases phosphorylation of protein kinase A and eNOS to increase $Ca^{2+}$-dependent NO production in endothelial cells and further, improves endothelium-dependent relaxation in wild type but not TRPV1 knockout mice [65]. Long-term administration of capsaicin also significantly alleviates atherosclerosis in apolipoprotein E-deficient mice via a TRPV1-dependent mechanism [66]. This effect could involve improved endothelium function as suggested above. However, capsaicin can also increase cholesterol efflux and reduce cholesterol uptake in vascular smooth muscle cells via calcium and protein kinase A-dependent mechanism(s). These effects of capsaicin on cholesterol transport/efflux were also found *in vivo* and translated to a significant reduction in aortic lipid load in mice on a high fat diet [66]. Further, capsaicin and other TRPV1 agonists have been shown to reduce lipid accumulation and pro-inflammatory cytokine responses in macrophages via the transcription factor, liver X receptor $\alpha$ (LXR$\alpha$) [67] thought to be involved in lipid and glucose homeostasis. Further, LXR$\alpha$ up-regulation by capsaicin may link the observed anti-inflammatory effects on macrophages to inhibition of NF-$\kappa$B and/or activation of the peroxisome proliferator-activated receptor $\gamma$ (PPAR$\gamma$) pathway [68].

In addition to favourable effects on the endothelium and cholesterol transport, capsaicin has also been suggested to be beneficial in metabolic syndrome and diabetes that can precede and/or contribute to cardiovascular disease. For example, uncoupling protein 2 (UCP2) that regulates mitochondrial ROS stress can be up-regulated by dietary capsaicin and is linked to decreased vascular oxidative stress in a diabetic mice model [69]. Capsaicin also appears to mitigate metabolic dysregulation as obesity-induced inflammatory responses of adipose tissue macrophages are reduced by capsaicin [70]. Further, decreased plasma glucose and lipid levels and pro-inflammatory genes are observed in obese diabetic mice exposed to dietary capsaicin [71] and in mice fed with a high fat diet after topical application of capsaicin [72].

Taken together, the cumulative evidence suggests that capsaicin and TRPV1 activation exert anti-inflammatory, anti-hypertensive and anti-atherosclerotic effects in the vasculature that can mitigate ischemic stress, cardiovascular disease

and cardiac dysfunction. Hence capsaicin has the potential to significantly limit cardiovascular damage and may be a novel natural cardioprotective agent.

### 4.3. Role in neuroprotection

Loss of neuronal function and structure can be caused by excitotoxicity, inflammation, oxidative stress, mitochondrial dysfunction and iron and protein accumulation. Interestingly, many of these causes are modulated by capsaicin either in neurones or in non-neuronal cells associated with the vasculature. As discussed above capsaicin shows considerable cardioprotective ability including mitigating ischemic injury, inflammation and vascular tone and this may be relevant to neuroprotection in the setting of stroke, neurodegeneration and central nervous system injury that share similar pathophysiological processes. Through agonism of TRPV1, capsaicin affects excitotoxicity and neuropeptide release, further suggesting consideration of its use as a neuroprotective agent.

A beneficial activity of capsaicin is observed in ischemic brain injury and stroke. For example, capsaicin mediates positive effects on cell viability via reduction of ROS and apoptosis in hippocampal neurones [73]. Increased cell survival in and around the infarct region after hypoxia-reoxygenation is associated with neuroprotection. This activity seems to contradict the well-known action of high dosage capsaicin in inducing ROS and $Ca^{2+}$-mediated mitochondrial damage and apoptosis that is particularly observed in cancer cells. However, it is also well-recognised that repeated exposure to capsaicin reduces the functionality of TRPV1, thereby modulating $Ca^{2+}$, neurogenic inflammation and related cell damage. Alternatively, capsaicin may exert other effects including acting as an antioxidant and direct action on cell signalling pathways and protein kinases. In any case, modulating inflammation and cell damage and increasing cell survival in stroke are likely to be beneficial.

Indeed, it has been suggested that capsaicin promotes cell survival via the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathway (Figure 3). Activation of this pathway was demonstrated by the accumulation of p-Akt in capsaicin pre-treated rat hippocampal neurones undergoing experimental stroke [73]. Another study

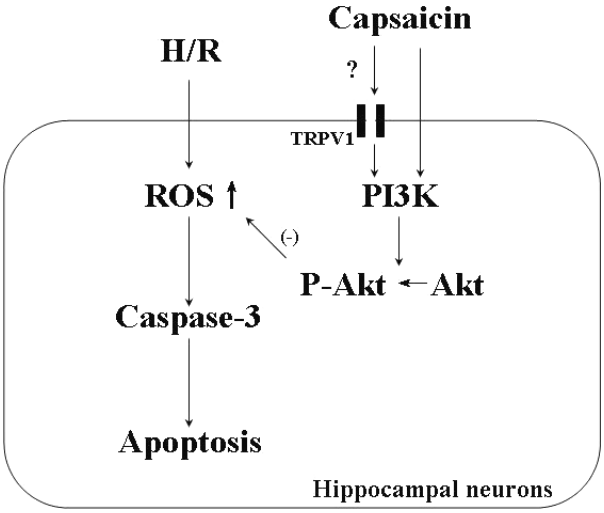


**Figure 3.** Schematic showing induction of PI3K by capsaicin. Capsaicin has been postulated to activate PI3K directly or via TRPV1. Activation of PI3K results in phosphorylation of Akt which inhibits ROS and apoptosis induced by experimental hypoxia/reperfusion (H/R) injury. (Schematic image taken from Guo, S. Y., Yang, G. P., Jiang, D. J., Wang, F., Song, T., Tan, X. H. and Sun, Z. Q. 2008, Can. J. Physiol. Pharmacol., 86, 785, © 2008 Canadian Science Publishing or its licensors. Reproduced with permission from the publisher).

confirmed p-Akt accumulation in prostate LNCaP cells following capsaicin treatment and implicated the MAPK/ERK signaling pathway suggesting that other anti-apoptotic kinases may be involved in neuroprotection afforded by capsaicin [74]. Also, it was further observed that both pathways were independently activated by capsaicin. However, other studies have shown a link between the PI3K and ERK pathways; there are conflicting reports as to whether PI3K increases or inhibits ERK activation [75].

Capsaicin activation of TRPV1 may participate in vascular autoregulation that is relevant for both cardiovascular protection and stroke although the vascular effects of receptor activity are complex. Capsaicin-mediated release of neuronal CGRP is considered vasodilatory. However, capsaicin can also induce smooth muscle cell contraction and vasoconstriction via release of substance P to regulate myogenic tone [76] and myogenic tone can be improved with capsaicin and is associated with neurovascular protection [77]. In addition to myogenic tone, capsaicin can improve endothelium-

dependent vasorelaxation, and dietary capsaicin has been demonstrated to relieve hypertension in spontaneously hypertensive rats [65]. Long-term administration of dietary capsaicin in spontaneously hypertensive rats reduces arterial hypertrophy and significantly delays stroke occurrence, and is associated with the activation of cerebrovascular TRPV1 and increased phosphorylation of eNOS [78].

It is also well known that mild hypothermia can reduce stroke injury and TRPV1-agonism has been shown to provide neuroprotection via this mechanism. For example, the TRPV1 agonist Rinvanil induces mild hypothermia that provides protection against cerebral ischaemia/reperfusion injury when provided pre- or post-occlusion [79]. This effect is prevented by capsazepine that blocks TRPV1 activity. Capsaicin also prevents neuropathological damage after global ischaemia in animals, via a TRPV1-mediated hypothermic effect and increased survival of pyramidal cells [80]. Further, dihydrocapsaicin administered post focal cerebral ischaemia induces hypothermia and improves function and decreases infarct size when used in a clinically relevant treatment regime [81]. In this study, the hypothermic and neuroprotective effects were lost with TRPV1 gene knockout suggesting that the stroke benefit is derived from TRPV1 agonism rather than off-target effects.

In contrast to the above, a recent report shows that TRPV1 gene knockout or receptor blockade with capsazepine improves neurological deficit and reduces infarct size in a mouse model of focal cerebral ischaemia [82] suggesting that TRPV1 can mediate neuropathological effects. There are also paradoxical reports of neurotoxic effects of capsaicin. For example, capsaicin can induce glutamate release [83] that can damage nerves cells and the brain via excitotoxicity. Further, activation of TRPV1 by capsaicin leads to degeneration of mesencephalic dopaminergic neurones *in vivo* and *in vitro* via increased intracellular $Ca^{2+}$ and mitochondrial damage [31]. There may also be neurotoxic crosstalk between TRPV1 and cannabinoid receptors (CB1) as they share common agonists, and a CB1 antagonist can mitigate capsaicin-induced neurotoxicity [31]. Glutamate toxicity is associated with both acute and chronic neurodegenerative disorders, and dopaminergic neurones are involved in motor control and learning suggesting that TRPV1 functions are important in neurodegenerative disorders.

However, there are also reports of capsaicin modulating excitotoxic damage. In studies of global cerebral ischaemia, capsaicin treatment markedly reduced or prevented cognitive and sensimotor deficits and this neuroprotection was associated with hypothermia and increased survival of pyramidal cells via TRPV1 [80]. It has also been proposed that exposure to high doses or repeated low doses of capsaicin can desensitise TRPV1 receptors, resulting in reduced $Ca^{2+}$ influx, glutamate release and subsequent excitotoxic damage [77]. Further, post-treatment with capsaicin or the phytochemical resveratrol, or a combination of both attenuated glutamate-induced toxicity in cerebral cortical neurones via significantly increasing cell viability and antioxidant genes and by decreasing apoptosis, ROS production and pro-inflammatory mediator (IL-1β and TNF-α) expression [84]. Interestingly, combined treatment with capsaicin and resveratrol enhances neuroprotection in an *ex vivo* model of neuronal excitotoxicity [84] suggesting that phytochemical combinations may provide therapeutic advantage in neurodegenerative disorders.

Capsaicin also appears to mitigate cognitive impairments and histopathological protein modifications that are reminiscent of Alzheimer's disease. Thus, capsaicin pre-treatment attenuated cold-water stress-induced dendritic retraction and synaptic damage via reinforcing the levels of several memory-associated proteins and by modulating tau hyperphosphorylation by increasing protein phosphatase 2 activity [85]. Thus the positive effects of capsaicin on hippocampal cell viability, hyperlocomotion and cognition noted in animal studies suggest that it may be therapeutically applied to chronic neurological diseases such as Parkinson's and Alzheimer's in addition to acute pathologies such as ischemic stroke. However, as suggested above, further study may be required regarding dosage and application of capsaicin considering its potential for paradoxical effects on regulation of TRPV1 in order to optimise neuroprotection rather than induce neurotoxicity.

## CONCLUSION

In summary, the available data suggests that modulation of TRPV1 receptors by capsaicin has the potential to provide benefit to several distinct pathological conditions linked to major organs such as the heart and the brain. Activation of TRPV1

receptors by capsaicin is linked to cell signalling pathways that are central to cell viability and survival and heightened oxidative stress and at least in the case of cancer, current evidence suggests that precancerous cells may react differently to capsaicin than non-cancerous cells. In terms of neuroprotective potential, capsaicin has been demonstrated to provide protection in both acute (*e.g*., ischemic stroke) and chronic (*e.g*., Alzheimer's disease and Parkinson's disease) injury models by reducing ROS production and inflammation, and inducing antioxidant responses. Capsaicin can be easily obtained, is well tolerated by humans and has the ability to cross the blood-brain barrier. These traits, in combination with its ability to provide protection make capsaicin a promising candidate in our quest to discover effective treatments for these increasingly prevalent neurodegenerative disorders.

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**CONFLICT OF INTEREST STATEMENT**

All authors have nothing to disclose.

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