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Receptor changes in brain tissue of rats treated as neonates with capsaicin

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ABSTRACT

Capsaicin, the hot chemical in chillies, administered to neonatal rats, causes destruction of polymodal nociceptive primary afferent neurons by acting on TRPV1 receptors causing intrinsic somatosensory deprivation. Although the effects of neonatal capsaicin treatment in the periphery have been extensively investigated, less is known about the brain networks to which the capsaicin sensory neurons are relayed. In the present study the effect of neonatal capsaicin treatment on brain receptors that have been shown to interact with TRPV1 was examined. Wistar rats were treated on neonatal day 2 with capsaicin and at 15–16 weeks of age, brains were processed to measure levels of muscarinic M_1/M_2 and M_2/M_4, serotonin $5HT_{2A}$, cannabinoid CB_1, dopamine D_1, D_2 receptors and dopamine transporter. Overall increases in levels of muscarinic M_1/M_4 ($F = 8.219$, df = 1, $p = 0.005$), muscarinic M_2/M_4 ($F = 99.759$, df = 1, $p < 0.0001$), serotonin $5HT_{2A}$ ($F = 28.892$, df = 1, $p < 0.0001$), dopamine D_1 ($F = 8.726$, df = 1, $p = 0.008$) and cannabinoid CB_1 ($F = 25.084$, df = 1, $p < 0.0001$) receptors were found in the brains of capsaicin-treated rats, although significant regional changes occurred only in muscarinic M_2/M_4 and serotonin $5HT_{2A}$ receptors. The results of the present study suggest that neonatal intrinsic somatosensory deprivation may have a significant impact on substrates at the central nervous system that manifest as changes in central cholinergic, monaminergic and cannabinoid systems in the adult animal.

1. Introduction

Capsaicin, the hot chemical in chillies, acts on transient receptor potential (TRP)V1 (vanilloid) receptors which belong to a large family of calcium-permeable cation channels (see Szallasi and Blumberg, 1999). TRPV1 receptors were first identified on a subset of primary afferent neurons involved in polymodal nociception and inflammatory responses (Caterina and Julius, 2001). However, they are now known to be widely distributed in the mammalian central nervous system although in much lower density than in the dorsal root ganglia (Mezey et al., 2000; Toth et al., 2005). TRPV1 mRNA and protein have been found in the cortex, in CA1–CA3 subfields of Ammon's horn and in the dentate gyrus of the hippocampus, thalamic and hypothalamic nuclei, basal ganglia, central amygdala, cerebellum, mesencephalon and hindbrain of rat and mouse brain (Mezey et al., 2000; Roberts et al., 2004; Cristino et al., 2006).

Capsaicin administered to neonatal rats, causes destruction of polymodal nociceptive primary afferent neurons which express TRPV1 receptors causing sensory deficits. Jancsó et al. (1977) were the first to demonstrate that administration of capsaicin to neonatal rats caused impaired nociception in response to chemical stimuli applied to the skin or eye. Subsequently, capsaicin-induced deficits in a number of sensory modalities subserved by small diameter unmyelinated fibres, including chemical thermal and mechanical nociception, have been widely reported (Jancsó et al., 1977; Cervero and McRitchie, 1981; Faulkner and Growcott, 1980).

Many of the receptor systems that are involved in nociception are also expressed in the central nervous system. For example, muscarinic M_2 receptors that mediate the major central muscarinic effects of tremor, hypothermia and analgesia, and serotonin $5HT_{2A}$ receptors that play a role in the affective assessment and cognitive modulation of pain (Kauer and Gibson, 2009), are widely expressed in the brain. Recently a role of TRPV1 receptors (the major target of capsaicin) in neurologic and psychiatric conditions has been suggested (see Chahl, 2007a; Di Marzo et al., 2008). TRPV1 receptors have been shown to interact with receptor systems implicated in psychosis such as the dopamine and cannabinoid CB_1 receptor systems. For example, in the basal

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ganglia TRPV1 immunoreactivity has been found in dopaminergic neurons (Mezey et al., 2000; Marinelli et al., 2007), and extensive co-localization of TRPV1 with cannabinoid CB_1 receptors has been found in the hippocampus, thalamus, hypothalamus and cerebellum of mouse brain (Cristino et al., 2006). In addition, several interactions with dopaminergic and cannabinoid systems have been reported (Tzavara et al., 2006; Starowicz et al., 2007; Micale et al., 2009). Furthermore, a role of TRPV1 receptors in synaptic plasticity in the hippocampus as well as in the superior colliculus during development has been proposed: TRPV1 knockout mice exhibit reduced hippocampal long-term potentiation (Marsch et al., 2007) whereas long-term depression at synapses on hippocampal interneurons in these mice is absent (Gibson et al., 2008). TRPV1 receptors have been shown to play a role in the mediation of long-term depression in the superior colliculus during development (Maione et al., 2009) suggesting that they may play a role in regulation of synaptic strength during brain development.

Degeneration of central axons and termination of peripheral sensory neurons lead to functional and structural alterations of substrates at spinal brainstem, thalamic and likely cortical levels (Wall et al., 2002). During postnatal development sensory experiences play critical roles in the refinement of cortical connections. Studies in developmental neurobiology have shown that neonatal somatosensory deprivation such as that induced by whisker trimming in the mouse whisker barrel model, results in reduced synaptic density in the barrel cortex (Sadaka et al., 2003). It has been suggested that given the plasticity of the nervous system in the newborn rat capsaicin induced degeneration may have a significant impact not only on the afferent nerves themselves but also on second and higher order neurons and related systems in the central nervous system as well as on systems associated with the peripheral endings of sensory neurons (Holzer, 1991). In agreement with this we have previously shown that neonatal capsaicin treatment (that presumably gives rise to an intrinsic somatosensory deprivation) leads to long-lasting neuroanatomical changes in the rat brain. For example, capsaicin-treated rats had reduced hippocampal and coronal cross-sectional area, reduced cortical thickness and increased neuronal density in several cortical areas, changes that are similar to those found in the brain of subjects with psychiatric disorders such as schizophrenia (Newson et al., 2005).

Although the effects of neonatal capsaicin treatment in the periphery have been extensively investigated, less is known about the brain networks to which the capsaicin sensory neurons are relayed. In view of the evidence given above the aim of this study was to determine the effects of neonatal capsaicin treatment on receptor systems in the central nervous system that are involved in nociception as well as in TRPV1 induced plasticity such as the cholinergic, monaminergic and cannabinoid systems in rat brain. Quantitative autoradiography with [^{3}H]pirenzepine, [^{3}H]AFDX384, [^{3}H] ketanserin, [^{3}H]CP55940, [^{3}H]SCH23390, [^{3}H]raclopride, and [^{3}H]GBR129354 was used to measure muscarinic M_1/M_4, muscarinic M_2/M_4, serotonin $5HT_{2A}$, cannabinoid CB1, dopamine D_1, dopamine D_2 receptors and dopamine transporter binding, respectively, in several brain regions of adult rats treated as neonates with capsaicin. We hypothesised that intrinsic somatosensory deprivation will affect brain development giving rise to regional/chemical abnormalities later in life that might be of relevance to psychiatric disorders.

2. Experimental procedures

2.1. Animals

Male and female Wistar rats were used in this study. All handling of animals and procedures were carried out in accordance with the guidelines established by the Animal Care and Ethics Committee of the University of Newcastle, an accredited research institution. Litters from two time-mated pregnant female rats were used. The rats were housed in plastic cages (73 cm × 54 cm × 24 cm) with shredded paper pellet bedding, and a wire mesh top cover. The animals were kept at a constant temperature of 21 ± 1 °C on a 12–12 h light–dark cycle with lights on at 0700 h. Food and water were freely available.

2.2. Drugs

The drugs used in this study were: capsaicin (Sigma–Aldrich Pty Ltd., Australia); sodium pentobarbitone (Lethabarb, Virbac (Australia) Pty Ltd., Australia); salbutamol sulphate aerosol (Ventolin, Allen & Hanburys, Australia). The stock solution of capsaicin, 10^{-2} M, was made in a vehicle of 10% Tween 80 and 10% ethanol in saline.

2.3. Capsaicin treatment

Capsaicin treatment was carried out as detailed in Newson et al. (2005). Briefly, 2 days after birth, newborn rats were individually removed from their home cage for subcutaneous injection of capsaicin (50 mg/kg) or vehicle, under ice anaesthesia. The duration of ice anaesthesia was less than 1 min. Following injection, neonates were placed in the observation chamber and a measured dose of salbutamol aerosol (Ventolin) was sprayed into the chamber to alleviate respiratory difficulty induced by capsaicin. Neonates were kept in the observation chamber until all signs of respiratory distress had disappeared. Rats appeared to suffer no ill effects from the hypothermia and the survival rate following capsaicin treatment was 95%. There was no local damage at the injection site in any of the animals. The rat pups were checked and weighed twice weekly. Rats were weaned 25–26 days after birth by placement into new cages, with one or two other animals of the same sex. At 15–16 weeks of age, rats were euthanased by intraperitoneal injection of sodium pentobarbitone, 100 mg/kg. Brains were removed immediately after death and frozen in isopentane. The brains were then transported to the Australian Nuclear Science and Technology Organisation (ANSTO) laboratories for further analysis. Coronal brain sections (16 μM) were cut using a cryostat, mounted onto microscope slides. The sections were taken from two brain compartments that correspond to Plates 12–15 (caudate nucleus level) and 28–32 (hippocampus level) from a standard rat brain atlas (Paxinos and Watson, 1986).

2.4. Receptor autoradiography

[^{3}H]AF-DX384 binding was performed based on the method used previously (Zavitsanou et al., 2005). Briefly, all sections were pre-incubated for 15 min at room temperature in 10 mM KH_2PO_4, 10 mM Na_2HPO_4 (pH 7.4). Sections were then incubated for 1 h in the same buffer containing 4 nM [^{3}H]AF-DX384 (specific activity 115 Ci/mmol, PerkinElmer, USA). Non-specific binding was determined by incubating adjacent sections in [^{3}H]AF-DX384 plus 10 μM atropine. Following incubation, sections were washed twice for 2 min each in room temperature buffer, and dipped once in distilled water and air dried.

[^{3}H]pirenzepine binding was performed based on the method used previously (Zavitsanou et al., 2004). The sections were pre-incubated for 15 min at room temperature in 22 mM HEPES (pH 7.4), and then incubated for 90 min at room temperature in the same buffer containing 10 nM [^{3}H]pirenzepine (specific activity 79 Ci/mmol, PerkinElmer, USA). Adjacent sections were incubated with [^{3}H]pirenzepine plus 10 μM of atropine to determine non-specific binding. After the incubation the sections were rinsed three times (4 min each) in ice-cold buffer with a final dipping in ice-cold distilled water and air dried.

Binding for $5HT_{2A}$ receptors was performed as previously described (du Bois et al., 2006) with minor modifications. Briefly, sections were pre-incubated for 5 min at room temperature in 170 mM Tris–HCl (pH 7.7). Sections were then incubated for 60 min at room temperature in the same buffer with the addition of 8 nM [^{3}H] ketanserin (specific activity 86.0 Ci/mmol, PerkinElmer, USA). Non-specific binding was determined by incubating adjacent sections in 8 nM [^{3}H] ketanserin in the presence of 10 μM spiperone. After this incubation, sections were washed twice for 15 min each in ice-cold buffer, followed by one dip in ice-cold distilled water and then dried.

For D_2 receptor binding slides were pre-incubated at room temperature for 20 min in a buffer containing 120 mM NaCl, 2 mM CaCl, 1 mM $MgCl_2$, 50 mM Tris–HCl (pH 7.4). Sections were then incubated in the same buffer with the addition of 4 nM [^{3}H] raclopride (specific activity 62.2 Ci/mmol, PerkinElmer, USA) for 60 min at room temperature in the absence or presence of 10 μM butactamol to determine the total and the non-specific binding, respectively. Post-incubation, sections were washed (2× 5 min) in ice-cold buffer and dipped in ice-cold distilled water and then dried.

D_1 receptor binding was carried out as detailed in Minuzzi et al. (2006). Slides were pre-incubated for 10 min at room temperature in a buffer containing 120 mM NaCl, 5 mM KCl, 2.5 mM CaCl, 1 mM MgCl, 50 mM Tris–HCl (pH 7.4). Sections were incubated in the same buffer with the addition of 3 nM [^{3}H] SCH 23390 (specific activity 85.0 Ci/mmol, PerkinElmer, USA) and 5 μM ketanserin for 60 min at room temperature. Non-specific binding was determined by incubating adjacent sections in the same incubation solution in the presence of 10 μM SKF 38393. Sections were