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SPECIAL REPORT

Cannabinoid inhibition of the capsaicin-induced calcium response in rat dorsal root ganglion neurones

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Cannabinoids have marked inhibitory effects on somatosensory processing, which may arise from actions at both peripheral and central cannabinoid receptors. Here, the effect of a synthetic cannabinoid agonist HU210 on capsaicin-evoked responses in adult rat dorsal root ganglion (DRG) neurones was studied. The vanilloid capsaicin produced a concentration-related increase in intracellular calcium in DRG neurones, which was significantly inhibited by HU210 (1 μM). The cannabinoid CB_1 receptor antagonist SR141716A (1 μM) had no effect alone and did not influence the response to capsaicin but significantly reversed the inhibitory effect of HU210. These data indicate that DRG CB_1 receptors are functional and can inhibit nociceptive responses.
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Abbreviations: $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; CB, cannabinoid; DRG, dorsal root ganglion; HU210, (6aR)-*trans*-3-(1,1-Dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol); SR141716A, (N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride); VR, vanilloid receptor

Introduction Recent studies indicate that cannabinoids have diverse effects on sensory nerve function. For example endogenous cannabinoids such as anandamide have vasodilator effects *via* an agonist action on sensory nerve vanilloid (VR_1) receptors (Zygmunt *et al*., 1999; Ralevic *et al*., 2000). However, both synthetic cannabinoids (Drew *et al*., 2000) and anandamide (Harris *et al*., 2000) inhibit C-fibre driven neuronal responses *in vivo via* cannabinoid$_1$ (CB_1) receptor activation. Nociceptive primary afferent fibres express both pro-nociceptive VR1 and anti-nociceptive CB_1 receptors and are therefore an ideal model for investigating interactions between these two receptor systems. Here we report the effect of the synthetic cannabinoid receptor agonist HU210 on capsaicin-evoked Ca^{2+} responses in adult rat dorsal root ganglion neurones (DRG) in primary culture.

Methods DRG were isolated from adult Wistar rats (200–300 g) and neurones cultured as described by Lindsay (1988) with minor modifications. Cells were grown on 13 mm glass cover slips for 24 h prior to incubation with Fura 2-AM (5 μM, 30 min, 37°C). The mean diameter of the cells sampled was 24.3 ± 0.8 μm. Intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in individual neurones in fields of 30–40 cells were estimated as the ratios of peak fluorescence intensities (measured at 500 nm) at excitation wavelengths of 340 and 380 nm respectively (Bundey & Kendall, 1999), using an Improvision imaging system. DRG neurones were superfused (2 ml min^{-1}) with different concentrations of the vanilloid receptor agonist capsaicin for 60 s, alone or in combination with the cannabinoid receptor agonist HU210 (1 μM) in the presence or absence of the cannabinoid CB_1 receptor antagonist SR141716A (1 μM), with 45 min wash-out periods between applications of capsaicin. Data are expressed as means $\pm$ s.e.mean. Statistical analysis was performed using one way ANOVA or Mann Whitney test.

Drugs HU210, (6aR)-*trans*-3-(1,1-Dimethylheptyl)-6a,7,10,-10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol) and capsaicin were purchased from Tocris Cookson Ltd. SR141716A (N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride) was provided by Research Biochemicals International as part of the chemical synthesis programme of the National Institute of Mental Health, Contract number N01MH300003. HU 210, SR141716A and capsaicin were dissolved in ethanol to a concentration of 10^{-2} M and stored at -30°C. Drug dilutions were made in superfusion buffer of composition (mM) NaCl 145; KCl 5; $CaCl_2$ 2; $MgSO_4$ 1; HEPES 10; glucose 10.

Results In untreated DRGs, the mean 340/380 nm ratio (reflecting basal $[Ca^{2+}]_i$) was 1.3 ± 0.03 ($n = 29$). Capsaicin produced a concentration-dependent increase in $[Ca^{2+}]_i$ (Figure 1) with an estimated EC_{50} value (calculated using GraphPad Prism) of 63 nM, ($n = 29$). Forty-five per cent of cells examined responded to capsaicin. There was little evidence of desensitization and a second exposure of the cells to 100 nM capsaicin 45 min after an initial challenge with the same concentration, produced a signal that was $85 \pm 6\%$ ($n = 26$) of the first response. In generating the concentration-response curve each of the cells was exposed to the full range of capsaicin concentrations. HU210 (1 μM) alone had no effect on $[Ca^{2+}]_i$ (Figure 2).

In the presence of HU210 (1 μM) peak responses to capsaicin (100 nM) were significantly reduced to $45 \pm 5\%$ of the control capsaicin response (Figure 3; $P < 0.001$, $n = 58$). SR141716A alone had no significant effect on capsaicin

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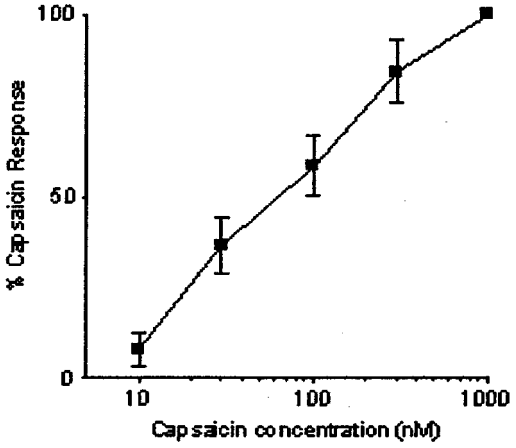

Figure 1 Increases in 340 : 380 nm ratios in single Fura 2 -loaded DRG neurones treated with capsaicin. Results are expressed as percentages of the peak responses to 100 nM capsaicin. Estimated $EC_{50} = 63$ nM ($n = 29$).

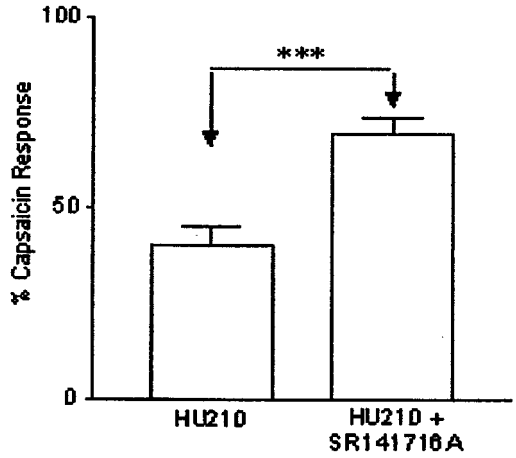

Figure 3 Inhibition of capsaicin responses in the presence of HU210 and reversal by SR141716A in individual DRG neurones. Results are expressed as percentages of the responses to 100 nM capsaicin alone. In the presence of HU210 (1 μM) the capsaicin response was reduced to $45 \pm 5\%$ of control ($P < 0.001$, $n = 58$). Co-administration of SR141716A (1 μM) partially reversed this to $70 \pm 4\%$ of control (***$P < 0.001$, Mann Whitney, compared with HU210 plus capsaicin.

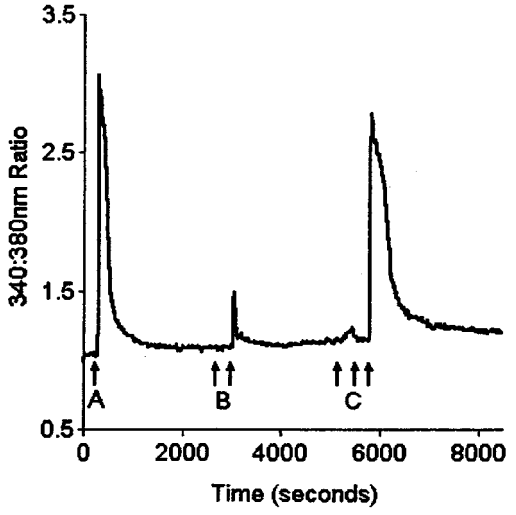

Figure 2 A representative trace showing changes in 340 : 380 nm ratios in a single DRG neurone, in response to capsaicin, in the absence or presence of HU210 and SR141716A. At point A, the cell was exposed to capsaicin (100 nM) for 60 s. Forty-five minutes later, at point B, HU210 (1 μM) was applied (first arrow) followed by capsaicin (second arrow) for 60 s. After another 45 min (C) SR141716A (1 μM) was applied (first arrow) followed by HU210 (1 μM, second arrow) followed by capsaicin (third arrow).

(100 nM)-evoked responses ($92 \pm 2\%$ of control capsaicin response, $n = 20$). Co-application of SR141716A (1 μM) partly reversed the inhibitory effect of HU210 (1 μM) on the capsaicin (100 nM)-evoked response ($70 \pm 4\%$ of control response, $n = 58$, Figure 3). The duration of the $[Ca^{2+}]_i$ response was also reduced by HU210 (1 μM) to $66 \pm 3\%$ ($n = 75$) of the control capsaicin response and was restored, in fact somewhat prolonged, in the presence of SR141716A (1 μM) to $116 \pm 6\%$ of the control response ($n = 75$).

Discussion In this study, capsaicin-evoked increases in $[Ca^{2+}]_i$ in adult DRG neurones have been employed to investigate interactions between VR1 and CB_1 receptors in sensory nerves. Our results corroborate previous electrophysiological studies of capsaicin-evoked responses (Helliwell *et al*., 1988), which are mediated by VR1 receptor activation (Caterina *et al*., 1997). We report here that the synthetic cannabinoid agonist HU210 inhibited capsaicin-evoked Ca^{2+} responses in DRG neurones. This effect of HU210 was largely reversed by the cannabinoid CB_1 receptor antagonist SR141716A. Thus, HU210 appears to modify VR1 responses indirectly through CB_1 receptor activation and it is unlikely that inhibition of capsaicin-evoked responses by HU210 arises as a result of a direct interaction with the VR1 complex. Although capsaicin responses in DRG neurones can desensitize in a Ca^{2+} and voltage sensitive fashion (Piper *et al*., 1999), there was little evidence of desensitization of responses in the present experimental protocol. In addition, effects of HU210 were readily reversible on washout, further suggesting that desensitization does not markedly contribute to these effects.

The present experiments support the existence of functional CB_1 receptors in DRG neurones and contrast a recent report that spinal CB_1 receptors are exclusively located at post-synaptic sites (Farquhar-Smith *et al*., 2000). Our functional data are in agreement with other, previously reported, expression studies of CB receptors in adult DRG neurones (Hohmann & Herkenham, 1999). Collectively the current body of evidence suggests that vanilloid and cannabinoid receptors are co-localized on the same sensory fibres.

Interestingly the endogenous cannabinoid anandamide is an agonist at both pro-nociceptive VR1 (Smart *et al*., 2000) and anti-nociceptive CB_1 receptors. Anandamide has a similar affinity for human VR1 and CB_1 receptors in model cell systems (low micromolar range; Smart *et al*., 2000; Rinaldi-Carmona *et al*., 1996) and increases Ca^{2+} signals to levels comparable to those produced by capsaicin (Smart *et al*., 2000). In contrast we report here that the synthetic cannabinoid agonist HU210 does not influence Ca^{2+} signals.

This finding is in keeping with previous reports that synthetic CB agonists such as WIN-55,212 and CP55,940 and antagonists such as AM281 and AM630 have no direct effect on VR1 receptors (Smart *et al*., 2000).

The present study provides strong evidence for a functional inhibitory role of pre-synaptic CB_1 receptors on adult DRG neurones. Our results strengthen the evidence that cannabinoids are antinociceptive *in vivo* (Drew *et al*., 2000), these effects arising, at least in part, from the activation of pre-synaptic CB receptors on sensory fibres. A recent study reported anandamide inhibition of capsaicin-induced bronchospasm *via* CB_1 receptor activation in the pulmonary sensory nerves (Calignano *et al*., 2000). This effect may arise from inhibition of excitatory transmitter release, as reported for CGRP release from vascular sensory nerves (Zygmunt *et al*., 1999), or inhibition of capsaicin-evoked VR1 receptor responses similar to those reported here.

Overall, there appear to be marked differences in the effects of endogenous versus synthetic cannabinoids on this sensory system expressing both VR1 and CB receptors, although the relevance of these differences in an intact physiological system remains unknown.

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