A cannabinoid agonist increases voltage-gated sodium current amplitude in differentiated neural progenitor cells by a mechanism that is independent of the transcription of SCNA genes

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Expression of voltage-gated currents (VGCs) is a key step in the development of excitability in neural progenitor cells (NPCs). Here we have studied whether the functional expression of VGCs during neuronal differentiation was influenced by the administration of a cAMP analogue, dibutyryl cAMP (DcAMP). The effect of WIN55,212-2 addition, a $CB_{1/2}$ receptor agonist, was also measured, since CB_1 and CB_2 receptors are negatively coupled to adenylate cyclase. NPCs were isolated from the ventricular zone of E14 Fischer 344 rats of either sex and cultured as a monolayer in 1µM FGF2 medium for 6 days. Differentiation was induced by FGF2 withdrawal (treatment 1) or, concomitant with FGF2 withdrawal, NPCs were exposed to 1 mM DcAMP (treatment 2) or 1µM WIN55,212-2 (treatment 3), with a vehicle control for WIN 55,212-2 (1 µl/ml acetone). NPCs were whole-cell voltage-clamped 2-4 days after treatment administration. Electrodes were filled with (mM) 133 KCl, 0.1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES and 10 EGTA, pH 7.4, tip resistances of 3-5 MΩ. Extracellular recording solution contained (mM) 132 NaCl, 3 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 5 HEPES and 5 glucose, pH 7·4. NPCs were held at -70 mV and VGCs were evoked during 50 or 500 ms steps to membrane potentials from -80 to +50 mV, increasing in 10 mV increments. Voltage-gated transient sodium (TS), transient potassium (TP) and persistent potassium (PP) membrane currents were recorded in all three treatment conditions. In addition, the total RNA was isolated from NPCs, and the mRNA levels for voltage-gated sodium channels (VGSC) were measured using real-time quantitative PCR. Primers were designed to detect all VGSC alpha subtypes; SCNA. Levels of SCNA mRNA were measured relative to mRNA for GAPDH. Data are shown in Table 1.

Table 1: Maximum membrane currents following FGF2 withdrawal alone or with DcAMP, WIN55,212-2 or acetone additions. Data in the right hand columns are mRNA expression levels relative to GAPDH. (Data are mean ± SEM; *P<0·05, **P<0·01, ANOVA with Dunnett's post-test, compared to FGF2-).

Treatment Condition	TS (nA)	TP (nA)	PP (nA)	n	SCNA mRNA	n
FGF2-	-2·1 ± 0·4	0·7 ± 0·1	1·5 ± 0·2	17	0·15 ± 0·05	8
FGF2- DcAMP+	-0·3 ± 0·3 *	0·5 ± 0·1	1·1 ± 0·2	11	0·11 ± 0·03	8
FGF2- WIN55,212-2+	-4·6 ± 1·3 **	1·2 ± 0·2 **	1·7 ± 0·3	13	0·14 ± 0·05	8
FGF2- Acetone+	-0·9 ± 0·4	0·4 ± 0·1	1·3 ± 0·2	7		

The data show that DcAMP significantly reduced TS activity, whilst WIN55,212-2 causes a marked increase in TS and TP activity, the latter of which is consistent with increased excitability. There was no change in the level of mRNA for VGSCs in either treatment group, suggesting that the increased VGSC was not due to increased transcription of SCNA genes. Since $CB_{1/2}$ receptors are negatively coupled to adenylate cyclase, these data are consistent with a role for receptor coupled control of ion channel expression in developing neurones, working via inhibition of adeylate cyclase. The regulatory mechanism does not involve regulation of transcription of SCNA genes but may be due to a post-translational regulation of VGSC activity or indeed regulation of transcription of other regulatory proteins