Blunting of CB_1-mediated effects in the striatum of rats overexpressing adenosine A_{2A} receptors

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An interaction between adenosine A_{2A} receptors (A_{2A}Rs) and cannabinoid CB_1 receptors (CB_1Rs) has been reported to occur in the striatum, where both receptors are highly expressed. Besides their main post-synaptic localization, striatal A_{2A}Rs are also localized on glutamatergic nerve terminals, as CB_1Rs, where they control glutamate release and glutamatergic neurotransmission.The modulation of CB1-mediated effects by A_{2A}Rs is rather complex, since the latter have been reported to exert both permissive and inhibitory effects (Tebano et al., 2012). To get further insight into such interactions, we verified whether the effects of CB_1R stimulation resulted altered in a condition of A_{2A}R overexpression. In particular we took advantage of TGR(NSEhA2A), a transgenic rat strain that overexpresses adenosine A_{2A}Rs (A_{2A}OE rats) in several brain areas including the striatum (Giménez Llort et al., 2007). In electrophysiology experiments, we evaluated the effect of the CB_1R agonist WIN55,212-2 (WIN) on synaptic transmission by recording extracellular field potentials (FPs) in corticostriatal slices from wild-type (WT) and A_{2A}OE rats. We found that WIN-induced reduction of synaptic transmission was significantly attenuated in A_{2A}OE animals compared to WT rats (64.58±2.83 and 29.99±3.48 of baseline, respectively, n=9). In both genotypes, the inhibitory action of WIN was reduced by the A_{2A}R agonist CGS21680. This result suggested a reduced functionality of CB_1R in the presence of an overexpression of A_{2A}Rs. To verify this *in vivo*, WT and A_{2A}OE rats were treated with WIN (2.5 mg/kg i.p.), and their locomotor activity was evaluated by automated cages. We found that WIN significantly reduced total locomotor activity in WT rats ($p<0.001$ *vs.* vehicle, n=7), while in A_{2A}OE rats the same dose of WIN was ineffective ($p=0.29$ *vs.* vehicle, n=7). As revealed by western blot experiments, the reduced functional activity of WIN did not depend on a reduced level of CB_1R protein in A_{2A}OE rats as compared to WT animals.The reduction of CB1-mediated effect in A_{2A}OE rats was further confirmed in synaptosomal preparation where the modulation by WIN of K^+-evoked glutamate release was reduced in A_{2A}OE rats compared to WT rats. Furthermore we found that CGS21680-induced increase of K^+-evoked glutamate efflux was significantly greater in synaptosomes obtained from A_{2A}OE rats ($p<0.05$ *vs*. WT) suggesting that in these animals an important overexpression of A_{2A}Rs occurs at the presynaptic level. To investigate this hypothesis we evaluated the *in vivo* effects of two A_{2A}Rs antagonists, SCH-442416 and KW-6002, which have been reported to discriminate between pre- and post-synaptic A_{2A}Rs, respectively (Orrú et al., 2011). In line with the overexpression of A_{2A}Rs, which negatively modulate motor activity, we found that spontaneous locomotor activity was significantly reduced in A_{2A}OE *vs*. WT rats (total counts over 60 min 3130±377 and 6096±850 respectively; $p<0.01$). The administration of both KW-6002 (a preferential 'post-synaptic' antagonist) and SCH44-442416 (the preferential 'pre-synaptic' antagonist) stimulated motor activity in habituated WT animals to a similar extent (2.8±0.9-fold and 7.0±2.6-fold increase, respectively), while in habituated A_{2A}OE rats acute administration of SCH-442416 induced a significantly greater stimulation than KW-6002 (33.5±7.5-fold and 9.8±3.5-fold increase, respectively; $p<0.01$). Our data clearly demonstrate that in presence of an overexpression of A_{2A}Rs, the functional effects elicited by CB_1R stimulation are blunted. Whether this depends on a relative predominance of pre-synaptic A_{2A}Rs and/or upon an unbalance between the proportion of A_{2A}Rs forming and not forming heteromers with CB_1Rs (Ferré et al 2010), remains to be determined.

Tebano et al. (2012) *Brain Res*. 1476:108-18; Gimenez-Liort et al. (2007) *Neurobiol Learn Mem*. 87:42-56; Orrù et al. (2011) *PLoS One* 6(1):e16088; Ferrè et al.(2010) *Br J Pharmacol*. 160(3):443-453.