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Modulation of glutamate release by a κ-opioid receptor agonist in rodent and primate striatum

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Abstract

The influence of the κ-opioid receptor agonist, enadoline, on endogenous glutamate release was investigated in rat and marmoset striatal synaptosomes. Enadoline decreased 4-aminopyridine (2 mM)-stimulated glutamate release (rat: IC_{50} ~ 8.7 μM, marmoset: IC_{50} ~ 2.9 μM). The effect of enadoline was reversed by nor-binaltorphimine (5 μM). These data indicate that, in the striatum of the rat and marmoset, κ-opioid receptor agonists can modulate glutamate release. These findings may have implications for the treatment of Parkinson's disease.

Keywords: Striatum; Glutamate; κ-Opioid receptor

The striatum is the major input structure of the basal ganglia, and therefore is important in the processing of information concerning motor control and cognition. The excitatory corticostriatal projection is overactive in Parkinson's disease (Carroll et al., 1995) and may be the key feature mediating the symptoms of Parkinson's disease. Glutamate release in the striatum can be influenced by inter alia dopamine (Maura et al., 1988), nitric oxide (Guevara-Guzman et al., 1994) and possibly glutamate itself (East et al., 1995). In this study, we used a striatal synaptosome preparation to establish whether activation of pre-synaptic κ-opioid receptors could also modulate glutamate release.

Synaptosomes were prepared from the striata of male Sprague-Dawley rats and the common marmoset as previously described (East et al., 1995). Briefly, synaptosomes were resuspended in Hepes-buffered medium (HBM; containing (in mM) NaCl (140), KCl (5), Hepes (20), $NaHCO_3$ (5), $MgCl_2$ (1), Na_2SO_4 (0.12), glucose (10)) and an aliquot (0.25–0.30 mg/ml protein, Bradford assay) added to a constantly stirred thermostated (37°C) cuvette with $NADP^+$ (1 mM), glutamate dehydrogenase (Sigma type III, 50 U) and $CaCl_2$ (1.3 mM) or EGTA (1.3 mM) in experiments involving nominally Ca^{2+}-free conditions. Glutamate release was monitored continuously during experiments using an enzyme-linked fluorometric assay in which glutamate-induced reduction of $NADP^+$ to NADPH produced an increase in fluorescence (excitation was at 340 nm and emission 460 nm). This change in fluorescence being proportional to glutamate concentration. 4-Aminopyridine (2 mM) was used to depolarise synaptosomes. Drugs were added 5 min prior to depolarisation. The duration of each experiment was 5 min, the first 80 s of the experiment are used to establish basal glutamate release, 4-aminopyridine was added at 80 s and any change in Ca^{2+}-dependent release is calculated by integrating the fluorescence signal with respect to time and comparing values before and after drug additions. In each experiment a concentration-response curve for glutamate (1–5 μM) and protein assay was established and used to convert fluorescence data to concentration (nmol glutamate/mg protein).

Incubation of synaptosomes with 4-aminopyridine (2 mM) produced an increase in Ca^{2+}-dependent glutamate release of 6.98 nmol glutamate/mg protein (n = 15) and 7.67 nmol glutamate/mg protein (n = 13) in rat and marmoset striatum respectively. Pre-incubation with enadoline produced a concentration-dependent inhibition of 4-aminopyridine-stimulated Ca^{2+}-dependent glutamate release in both rat and marmoset. Maximum inhibition occurred at 300 μM enadoline

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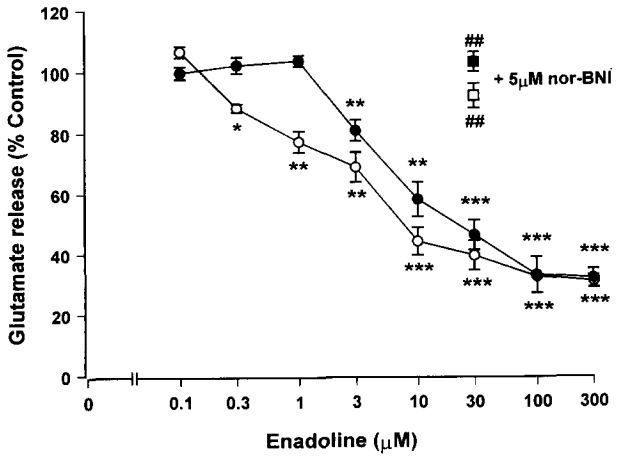

Fig. 1. Inhibition of 4-aminopyridine-stimulated Ca^{2+}-dependent glutamate release from rat (closed circles) and marmoset (open circles) striatum by the κ-opioid receptor agonist enadoline. Enadoline or nor-binaltorphimine (nor-BNI) was applied to the synaptosomes 5 min prior to addition of 2 mM 4-aminopyridine. Significant inhibition was observed with enadoline ($^{*}P < 0.01$, $^{**}P < 0.005$, $^{***}P < 0.002$ compared to 4-aminopyridine alone; ANOVA followed by Duncan's post-hoc test; data shown are the mean ± S.E.M., $n = 3$–6 experiments). nor-BNI (5 μM) significantly inhibited the 30 μM enadoline-induced inhibition in both rat (closed square) and marmoset (open square) striatum ($^{\#\#}P < 0.005$ compared to 4-aminopyridine + 30 μM enadoline; ANOVA followed by Duncan's post-hoc test; data shown are the mean ± S.E.M., $n = 3$ experiments). Control release (100%) was 6.98 nmol glutamate/mg protein ($n = 15$) and 7.67 nmol glutamate/mg protein ($n = 13$) in rat and marmoset striatum respectively.

(rat: 68%; marmoset: 69%) and the IC_{50} was approximately 8.7 μM and 2.9 μM in rat and marmoset respectively. Glutamate release in the presence of enadoline (30 μM) and nor-binaltorphimine (5 μM), a selective κ-opioid receptor antagonist, was not significantly different from control in either rat or marmoset (Fig. 1). However, pre-incubation with cyprodime (20 μM) or naltrindole (10 μM), selective μ-opioid and δ-opioid receptor antagonists respectively, had no effect on the enadoline-induced inhibition of glutamate release (data not shown).

These results show that activation of pre-synaptic κ-opioid receptors inhibits glutamate release from terminals within the rat striatum. The 3-fold difference in potency of enadoline between rat and marmoset may indicate differences in κ-opioid receptors between species. The endogenous ligand for the κ-opioid receptor is dynorphin and mRNA encoding for this peptide is present in the striatum. Indeed, dynorphin is co-transmitted with GABA in recurrent collaterals in the striatum (Gerfen and Young, 1988). We have previously demonstrated that intraperitoneal administration of κ-opioid agonists has anti-parkinsonian effects in rat and marmoset models of Parkinson's disease (Mitchell et al., 1995; Maneuf et al., 1995). In addition, we have demonstrated that κ-opioid agonists reduce depolarisation-evoked glutamate release from rat substantia nigra slices (Maneuf et al., 1995), indicating a site of action of κ-opioid agonists in the output regions of the basal ganglia. Here we demonstrate that activation of κ-opioid receptors may also modulate functioning of the major input nucleus of the basal ganglia, the striatum.

In Parkinson's disease there is functional overactivity of the corticostriatal pathway. In addition dynorphin transmission is reduced in the parkinsonian patient (Engber et al., 1991) and our results suggest that this reduction may contribute to this overactivity. It has been demonstrated that modulating post-synaptic glutamate receptors within the striatum can produce anti-parkinsonian effects (Carroll et al., 1995). Our results suggest that pre-synaptic activation of κ-opioid receptors may be beneficial in the treatment of Parkinson's disease, by reducing glutamate release from the overactive corticostriatal terminals.

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