Reversal of tolerance to morphine by ethanol: role of PKC inhibition.

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Ethanol is detected in approximately 50% of heroin related overdose deaths (Hickman *et al.*, 2008). This suggests that ethanol may contribute to the dangers of opioid overdose. We have sought to examine whether ethanol can reduce tolerance to opioids thus increasing the risk of overdose. PKC has been implicated in MOPr desensitisation and cellular tolerance to morphine (Bailey *et al.,* 2009). To investigate the interaction between ethanol and cellular tolerance at the level of the μ-opioid receptor (MOPr) we measured MOPr function in rat brain neurones and examined whether ethanol inhibited protein kinase C (PKC) activity.

Whole cell voltage clamp recordings (Vh = -60 mV) were made from individual locus coeruleus (LC) neurones in rat brain slices as described previously (Bailey *et al*., 2009). MOPr activation evoked a transmembrane K^+ current, the amplitude of which is a measure of MOPr function. Brain slices were prepared from rats pre-treated with morphine for 3 days prior to sacrifice and maintained at 33-34℃ in morphine (1 μM). Tolerance was assessed by measuring the response to a maximally effective concentration of morphine (30 μM) and comparing that to the maximum response to noradrenaline in the same neurone (Bailey *et al*., 2009). A nonradioactive PKC detection kit (PepTag assay kit; Promega) was used to measure kinase activity *in vitro* according to the manufacturer's instructions. Recombinant PKCα protein (10 ng) in lipid vesicles containing varied amounts of diacylglycerol (DAG; 0%, 2%, 4% or 8%) were prepared and enzyme activity determined in the presence and absence of ethanol by incubation with Peptag C1 substrate peptide for 30 min at 30℃. Phosphorylated peptide was resolved by SDS-PAGE and quantified by densitometry after detection by UV illumination.

When LC neurones were exposed to a low concentration of ethanol (20 mM) for 10 min prior to and during the morphine challenge cellular tolerance was reversed (Table 1). This was not a direct effect of ethanol to potentiate current through the GIRK channels as 20 mM ethanol did not potentiate the acute response to morphine in neurones taken from non-morphine pre-treated animals.

Table 1. Maximum response to morphine in LC slices from naïve and morphine treated rats in the absence and presence of ethanol

Treatment	Maximum morphine response (% maximum response to noradrenaline)
Naïve	103 ± 7
Naïve + ethanol 20 mM	110. ± 7
Morphine treated	68 ± 2.5*
Morphine treated + ethanol 20 mM	110 ± 8.5†

(*p<0.01 compared to Naïve; † p<0.01 compared to morphine treated; ANOVA followed by Bonferroni test; n = 4 – 6)

As PKCα has been implicated in morphine tolerance in LC neurones (Bailey et al., 2009) we investigated the effects of ethanol on PKCα activity. 100 mM ethanol produced a 16.8 ± 2.4 % and 21.0 ± 2.6 % decrease in PKCα activity in vesicles containing 4% and 8% DAG respectively (n = 5,

$p<0.001$ compared to control, ANOVA followed by Dunnett's test). However, at 20 mM ethanol did not reduce PKCα activity with any of the DAG concentrations investigated.

These data demonstrate that ethanol reverses opioid cellular tolerance. Although PKCα is involved in cellular morphine tolerance we cannot conclude that ethanol reverses tolerance by inhibiting PKCα as ethanol only inhibited kinase activity at a high concentration. The interaction between ethanol and PKCα appears to be dependent on the concentration of DAG present.

References

Bailey CP, et al., (2009) Eur J Neurosci. 29:307-318

Hickman M, et al., (2008) Addiction. 103:1060-1062.