CELLULAR TOLERANCE TO MORPHINE IN ADULT RAT NEURONES

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In vivo, tolerance to morphine can occur rapidly, even after a single dose. However, in rat locus coeruleus neurones morphine, unlike other μ-opioid receptor (MOR) agonists, does not produce rapid MOR desensitization over the course of 30 min exposure to the drug (Alvarez *et al*., 2002; Bailey *et al*., 2003). We have used a protocol that enables us to study tolerance caused by long-term (6-9 hrs) morphine treatments, *in vitro*.

Whole cell patch clamp recordings (Vh –60mV) were made from visually identified locus coeruleus (LC) neurones in 250 μm thick pontine brain slices prepared from male Wistar rats (120-150g) as described previously (Bailey *et al*., 2003). Activation of MORs results in opening of G-protein coupled inwardly-rectifying K^+ channels (GIRK), that provides a real-time measure of the level of MOR activation. Slices were incubated in morphine (1 or 30 μM) for 6-9 hours, and the following drugs were sequentially applied: 30 μM morphine, 10 μM DAMGO, 1 μM naloxone (a competitive MOR antagonist) and 100 μM noradrenaline (NA). As the concentrations of morphine, DAMGO and NA are receptor saturating, the maximum responses to each drug can be obtained. In the rat LC, morphine is a partial MOR agonist, DAMGO is a full MOR agonist, and NA, through α_2 receptors, activates the same population of GIRKs as MOR (North & Williams, 1985). In this way, moderate tolerance would be seen as a decrease in max morphine/max DAMGO ratio, with no decrease in max DAMGO/max NA ratio. A high level of tolerance would result in decreases in both ratios.

Table 1. Ratios of maximum responses to morphine/NA and DAMGO/NA following 6-9 hr drug treatments. n = 3-5. * = P < 0.05 Student's t-test vs. control.

	Morphine/NA max ratio	DAMGO/NA max ratio
Control	94 ± 13 %	150 ± 15 %
1 μM morphine (6-9 hrs)	79 ± 5 %	137 ± 11 %
30 μM morphine (6-9 hrs)	29 ± 6 % *	66 ± 3 % *
1 μM morphine + 1 μM PMA	45 ± 10 % *	126 ± 10 %
1 μM PMA alone	89 ± 11 %	149 ± 15 %

1 μM morphine caused no tolerance, whereas 30 μM morphine caused a high degree of tolerance as both the morphine/NA and DAMGO/NA max ratios were significantly decreased (Table 1). As we have previously shown that activation of protein kinase C (PKC) can cause rapid (<7min) MOR desensitisation by morphine (Bailey *et al.,* 2004), we next examined whether PKC activation could reveal tolerance following 1 μM morphine, by concomitant application of the phorbol ester PMA (1 μM). These data show that in the presence of PMA, 6-9 hour incubation with morphine, even at the low concentration of 1 μM, causes tolerance. These findings show cellular tolerance to morphine in adult mammalian neurones, and highlight the critical role of PKC in morphine tolerance.

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