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Morphine and clonidine activate different K^+ channels on rat amygdala neurons

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Abstract

In cell-attached patch-clamp recordings from freshly dissociated neurons of the rat amygdalohippocampal area, clonidine principally activated a 95-pS (picosiemens) inwardly rectifying K^+-permeable channel, whereas morphine acting at μ-opioid receptors activated a different 130-pS channel. Clonidine's effects were largely antagonized by the α_2-adrenoceptor antagonist idazoxan, but were poorly mimicked by agmatine. These results partially contradict the prevailing hypothesis that α_2 and opioid receptors act through the same ion channel transduction pathways.

Keywords: Amygdala; Opiate; Clonidine

The α_2-adrenoceptor agonist clonidine is used clinically to reduce the symptoms of opiate withdrawal and to enhance opiate analgesia (Gold et al., 1978; Fairbanks and Wilcox, 1999). Locus coeruleus neurons are inhibited by both μ-opioid receptors and α_2-adrenoceptors (Aghajanian, 1978), and it has been suggested that both receptors activate the same K^+ channels (Miyake et al., 1989). Similarly, in amygdala neurons, inhibitory responses to opioids and to clonidine are highly colocalized to the same neurons (Freedman and Aghajanian, 1985). Thus, it is widely believed that opiates and clonidine can act through different receptors, but share the same downstream transduction pathways to account for opiate–clonidine functional interactions on the same cells. We have tested this hypothesis in neurons from the rat amygdala, where we have recently described a 130-pS inwardly rectifying K^+-permeable channel that is activated by μ-opioid receptors in a membrane-delimited manner (Chen et al., 2000).

Neurons were freshly dissociated from the amygdalohippocampal area and cell-attached patch recordings were performed as previously described (Chen et al., 2000). When clonidine (400 nM or 5 μM) was applied via the patch pipette, the 130-pS channel was observed in three out of 59 (~5%) patches (Fig. 1A), and was indistinguishable from channels previously seen in recordings with opioid agonists (Chen et al., 2000). However, we previously found that morphine activated this channel in >30% of patches from these same cells (Chen et al., 2000). In contrast, we observed a 95-pS channel in 15 out of 59 (~25%) recordings with clonidine (Fig. 1B), and this channel was never observed in the presence of opioids or in the absence of drugs (Chen et al., 2000). The 95-pS channel carried inward currents (with 140 mM KCl as the principal charge carrier) with no obvious voltage-sensitivity of activation at voltages negative to the reversal potential, and outward currents were not detected at more positive voltages (Fig. 1B). Thus, the 95-pS channel did not differ appreciably in its ionic properties or inward rectification from the 130-pS channel. However, their single channel conductances were unambiguously different, showing them to be distinct molecules (Fig. 1C).

Of the various channels expressed by these cells under these conditions, the 95-pS channel appeared to be the principal one associated with the presence of clonidine (Fig. 1D), and most of the others were spontaneously expressed in the absence of drugs. When the α_2-adrenoceptor antagonist idazoxan was applied along with clonidine, 95-pS channel activation was largely antagonized ($P < 0.05$, χ^2; Fig. 1D). Clonidine can also act at receptors for agmatine (Reis and Regunathan, 2000). However,

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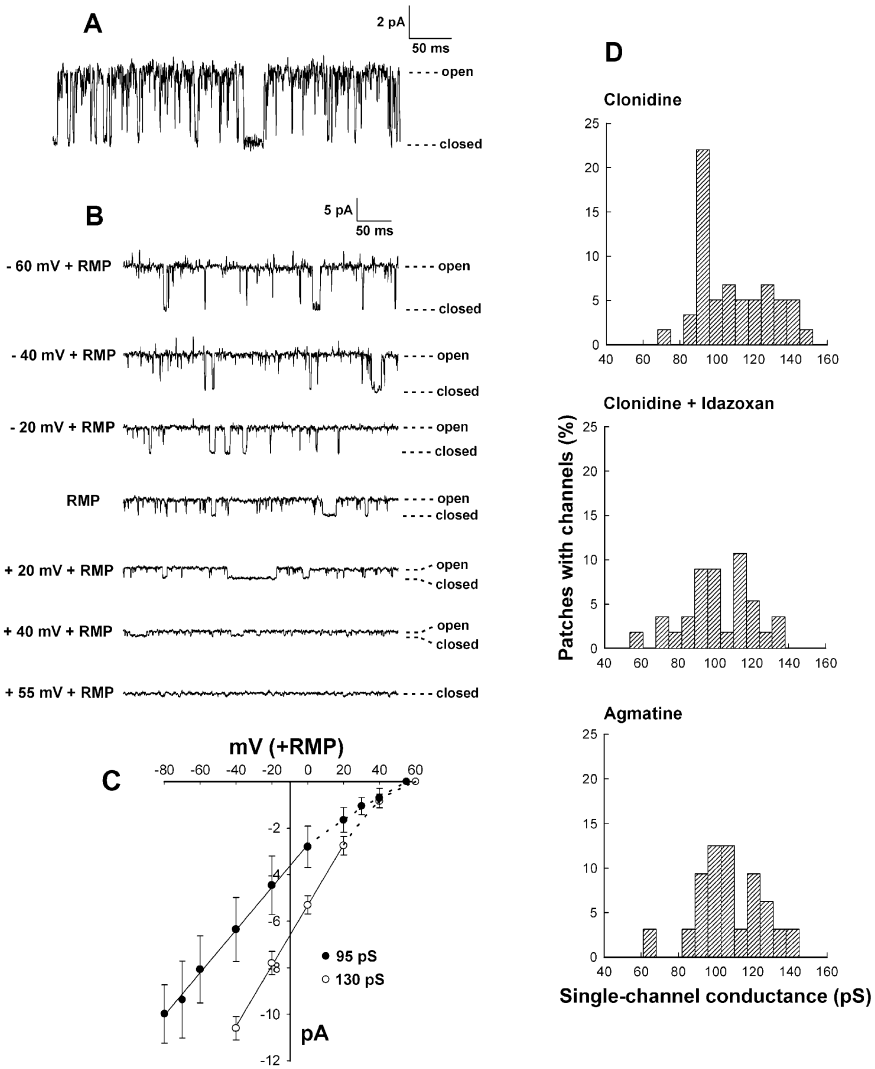

Fig. 1. (A) Recording of a 130-pS channel with 5 μM clonidine present in the patch pipette. The recording was made at resting membrane potential, and upward deflections correspond to inward currents. (B) Recordings of a 95-pS channel, also with 5 μM clonidine present. Recordings are shown at various voltages, expressed relative to the resting membrane potential (RMP). (C) Current–voltage relationships. Data are expressed as mean ± S.D. for 5–15 recordings at each point. For the 130-pS channel, the patch pipette contained 10 μM morphine. The solid lines were fitted by linear regression, giving the indicated conductances from their slopes. Dashed lines connect the points < 2 pA, where rectification occurred and which were not included in the fit. (D) Percentages of patches expressing channels of various conductances. The numbers of patches displaying channels of a given conductance range are expressed as the percentage of all patches recorded, and one patch was tested per cell. Top, pooled data for 400 nM and 5 μM clonidine, $n = 59$ cells. There was no difference in occurrence between the two concentrations. Middle, data for 400 nM clonidine plus 2 μM idazoxan, $n = 56$ cells. Bottom, data for 100 μM agmatine, $n = 32$ cells.

agmatine was not very effective at mimicking clonidine ($P > 0.5$ vs. clonidine plus idazoxan; Fig. 1D). Clonidine, thus, was acting primarily through α_2-adrenoceptors on these cells.

By performing single-channel recordings, we have been able to differentiate between the 95- and 130-pS channels. Our results partially contradict the prevailing model that α_2 and opioid receptors activate the same ion channels in the brain. Instead, at least in this limbic region of the brain, the two receptors act principally through different ion channels to elicit similar changes in membrane excitability on the same cells. The smaller amount of shared effects on the 130-pS channel are compatible with the coupling patterns of the two receptors to G-protein subtypes recently described in spinal neurons (Karim and Roerig, 2000). Clonidine and opiates do not display cross-tolerance (Fairbanks and Wilcox, 1999). Our results thus suggest a subcellular mechanism for how clonidine can increase opiate analgesia and decrease opiate withdrawal without being subject to cross-tolerance.

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