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Effects of the β-amino acid antagonist TAG on thalamocortical inhibition

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ABSTRACT

Chemical transmission at inhibitory synapses in thalamus may involve receptor activation by β-amino acids and glycine, as well as GABA. Given their hypothesized roles, we investigated effects of the putative β-amino acid antagonist 6-aminomethyl-3-methyl-4H-1,2,4-benzothiadiazine-1,1-dioxide (TAG) on synaptic inhibition in dorsal thalamus. We performed whole-cell recordings in 200–250 μm sections and immunocytochemical (ICC) studies in ventrobasal thalamus of rat brain (P12-P14). Stimulation of medial lemniscus evoked inhibitory postsynaptic currents (IPSCs) which were purely glycinergic or $GABA_A$ergic, or most commonly mixed glycinergic and $GABA_A$ergic responses, based on abolition by strychnine, bicuculline, or combined antagonism. TAG antagonized mixed IPSCs (IC_{50} ~70 μM) in a manner distinguishable from classical glycine and $GABA_A$ receptor antagonists. TAG (250 μM) reduced the amplitude of glycinergic components which had a decay time constant of ~9 ms or ~230 ms by 45–50%, and a $GABA_A$ergic component which had a decay time constant of ~40 ms by ~60%. As in the glycinergic component, TAG reduced the amplitude of infrequently occurring, pure glycinergic IPSCs. Surprisingly, TAG had no effect on pure $GABA_A$ergic IPSCs, with a decay time constant of ~20 ms that correlated to kinetics of GABA-activated channels. ICC studies showed co-localization of $\alpha_{1/2}$ glycine and α_4 $GABA_A$ receptors at inhibitory synapses. Activation of α_4 receptors by β-amino acids may contribute to the $GABA_A$ergic component of mixed IPSCs. The short and long-duration glycinergic IPSCs had decay time constants that correlated to the burst durations of single channels opened by β-amino acids and glycine. Overall, the effects of TAG implicate β-amino acid involvement in $GABA_A$ergic and glycinergic transmission.

1. Introduction

In the central nervous system, receptor antagonism is instrumental for elucidating the nature of the neurotransmitter that mediates synaptic inhibition. For issues of co-mediation by γ-aminobutyric acid (GABA) and glycine, the selectivities of antagonists are critical for identifying inhibitory postsynaptic currents (IPSCs). In mixed IPSCs, bicuculline or gabazine antagonizes the $GABA_A$ergic component and not glycine receptors, whereas the reverse is true for strychnine which only blocks the glycinergic component (Jonas et al., 1998; Dumoulin et al., 2001). Combined $GABA_A$ and glycine receptor antagonism eliminates the mixed IPSCs, frequently observed in thalamic neurons, which less commonly display pure $GABA_A$ergic and pure glycinergic IPSCs (Ghavanini et al., 2005; cf. cerebellum, Dumoulin et al., 2001).

The observed biophysical characteristics of mixed and pure IPSCs may result from precise receptor subtypes that play a crucial role in transmitter agonist recognition and kinetic properties (cf. Takahashi et al., 1992; Keramidas and Harrison, 2008). Thus, the $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor subtype which is abundant at forebrain synapses (cf. Farrar et al., 1999) likely mediates the pure $GABA_A$ergic IPSCs that decay with a time constant of ~22 ms (Ghavanini et al., 2006). Although the presence of the δ subunit often results in receptor expression in the extrasynaptic membrane, atypical $GABA_A$ receptors of composition $\alpha_4\beta_2\delta$ (Korpi et al., 2002) likely occur in synapses on ventrobasal neurons (Jia et al., 2008).

The extent to which α_4 $GABA_A$ receptors determine the pharmacological and biophysical properties of mixed and pure IPSCs is presently unclear. Previous studies have shown that $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta_2\delta$ $GABA_A$ receptors differ in their pharmacological properties (Jia et al., 2005). Taurine and β-alanine are full agonists at $\alpha_4\beta_2\delta$ receptors (Jia et al., 2008), but are partial agonists at $\alpha_1\beta_2\gamma_2$ receptors (cf. Wu et al., 1993; Hussy et al., 1997). The observations

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suggest that these β-amino acids may activate α_4 $GABA_A$ receptors in producing mixed IPSCs.

In view of previous studies, we also considered the possibility that the endogenous β-amino acids, in addition to glycine itself, may activate glycine receptors in the mixed IPSCs. Endogenous β-amino acids activate glycine receptors on neurons of the hippocampus (Mori et al., 2002), nucleus accumbens (Jiang et al., 2004), and amygdala, (McCool and Botting, 2000). The glycinergic component decays rapidly or slowly (Ghavanini et al., 2006). In this component, the kinetically distinct currents likely reflect two receptor populations that contain α_1 glycine subunits (faster decay) or co-assembled α_1 and α_2 subunits (slower decay, cf. Takahashi et al., 1992; Singer and Berger, 1999). The faster decay correlates to short-duration channel bursts induced by glycine, taurine and β-alanine. The slower decay correlates to long-duration bursts induced mainly by the β-amino acids. On these grounds, therefore, β-amino acids may contribute to the glycinergic component of mixed IPSCs and to pure glycinergic IPSCs.

If available, a selective β-amino acid antagonist would facilitate analysis of inhibition in the thalamus. Early studies on the role of taurine as a transmitter resulted in discovery of the putative antagonist, TAG (6-aminomethyl-3-methyl-4H-1,2,4-benzothiadiazine-1,1-dioxide (Yarbrough et al., 1981; Girard et al., 1982). In central neurons, TAG antagonized the firing depression induced by β-amino acids, and not by glycine or GABA (Okamoto et al., 1983; Padjen et al., 1989; Billard and Batini, 1991). This selectivity of TAG for receptors activated by taurine and β-alanine was compatible with its antagonism of β-amino acid binding in tissue homogenates (Martin et al., 1981; Frosini et al., 2003). While these actions likely involved extrasynaptic receptors, there is little information about TAG actions on synaptic currents.

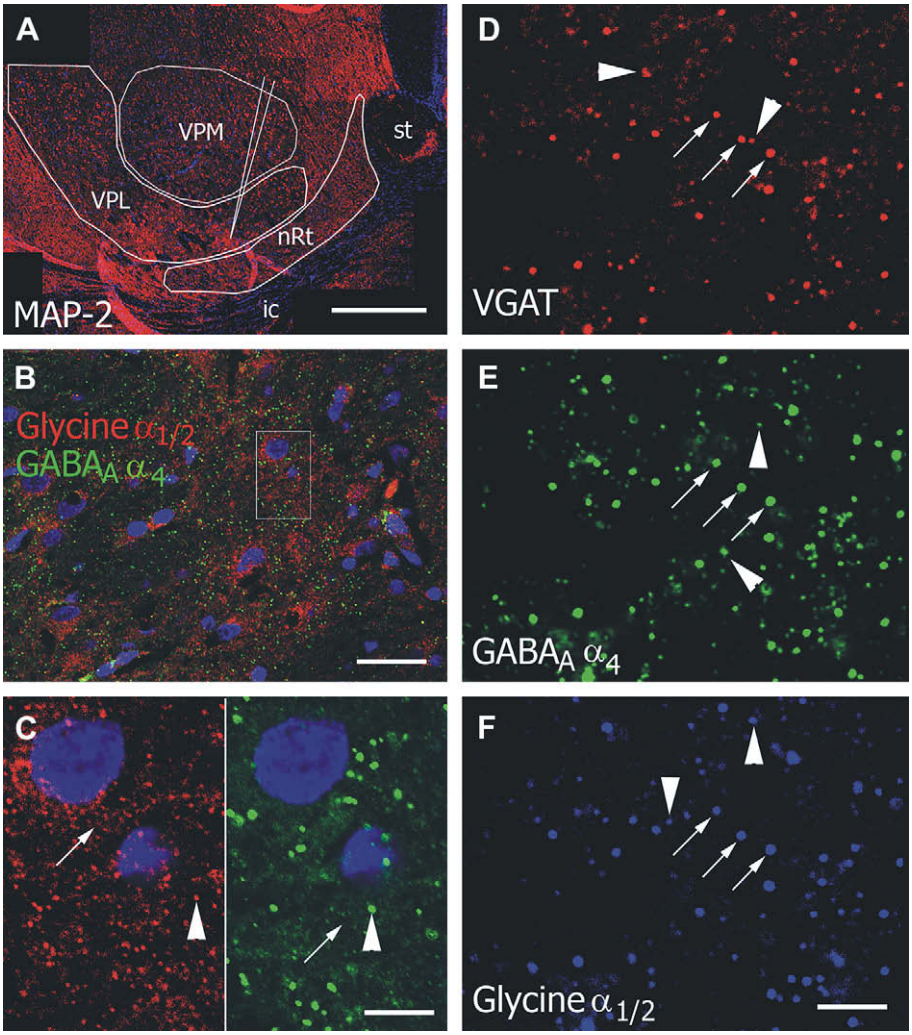

Fig. 1. Co-localization of synaptic glycine and $GABA_A$ α_4-containing receptors in VPL. (A) Low-power epifluorescence photomicrograph montage showing sagittal section of rat thalamus (P12) stained with antibody to MAP-2 (red), with nuclei counter-staining using DAPI (blue). Ventroposterolateral (VPL), ventroposteromedial (VPM), and reticular (nRt) nuclei of the thalamus are outlined. Note that MAP-2 staining was absent in the white matter of the stria terminalis (st) or the internal capsule (ic). The large V points to the VPL region used for recording and detailed immunocytochemical studies. (B) Confocal image of this region in another slice, stained for $GABA_A$ receptor α_4 subunits (green) and glycine receptor $\alpha_{1/2}$ subunits (red) with DAPI nuclear staining (blue). Co-localized staining for the two receptor subunits is indicated by yellow puncta. (C) High power confocal images of the neurons in the box in (B), stained for glycine receptor $\alpha_{1/2}$ subunits (red, left) and $GABA_A$ α_4 subunits (green, right). Punctate (large arrow heads) and diffuse (small arrows) staining was evident for both receptor subunits in somatic regions. (D–F) In a further slice, a field similar to that in (C) is shown at high magnification, stained using antibodies for (D) VGAT (red), (E) $GABA_A$ α_4 subunits (green) and (F) glycine receptor $\alpha_{1/2}$ subunits (blue), captured with a confocal microscope. White arrows in D–F indicate where staining for all three antigens overlapped, showing co-localization. Arrowheads indicate where staining was positive for the specified antigen but negative for the remaining two, showing no co-localization. Scale bars: (A) 500 μm, (B) 15 μm, (C and F) 5 μm. Bar in F applies to D–F. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)