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RESEARCH ARTICLE

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Modulation of sensory inhibition in the ventrobasal thalamus via activation of group II metabotropic glutamate receptors by 2*R*,4*R*-aminopyrrolidine-2,4-dicarboxylate

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Abstract Recordings were made from single neurones responsive to somatosensory input in the ventrobasal thalamus of the anaesthetised rat. GABAergic afferent inhibition arising from the thalamic reticular nucleus was evoked using a condition-test vibrissal stimulation paradigm. Local iontophoretic application of the group II metabotropic glutamate receptor (mGluR) agonist 2*R*,4*R*-4-aminopyrrolidine-2,4-dicarboxylate (2*R*,4*R*-APDC) in the vicinity of the recorded neurones produced a reduction of the afferent inhibition (from 78±3.0% to 25±5.3%), presumably via a presynaptic mechanism. This effect could be antagonised by LY307452, a known group II mGluR antagonist. In contrast, two selective group I mGluR agonists, (*S*)-3,5-dihydroxyphenylglycine (DHPG) and *trans*-azetidine-2,4-dicarboxylate (*t*ADA), were without effect on the GABAergic inhibition. These data show that group II but not group I mGluRs can have a significant role in the modulation of GABAergic afferent inhibition in the ventrobasal thalamus. This could be of importance in the control of sensory discriminative processes and functions of sleep, arousal and seizure generation.

Key words Metabotropic glutamate receptors · 2*R*,4*R*-4-Aminopyrrolidine-2,4-dicarboxylate · Somatosensory thalamus · Presynaptic inhibition · Rat

Introduction

The ventrobasal thalamus (VB) is the primary relay nucleus for somatosensory information ascending to the cerebral cortex. Modulation and gating of sensory transmission can occur at this level via a number of different mechanisms (Jones 1985; McCormick and Bal 1994). One such gating mechanism is GABAergic inhibition of the relay neurones in the VB. In the rat, a simple recurrent inhibitory circuit is present: this originates from GABAergic neurones in the adjacent thalamic reticular nucleus (nrt), which send axons into the body of the VB. This inhibitory circuit is activated by collaterals from VB relay neurone axons en passant to the cerebral cortex and by an excitatory input from layer VI of the cerebral cortex itself. The system is topographically organised so that it can mediate surround inhibition, and this makes this system important in the spatial and temporal discrimination of stimuli within VB (De Biasi et al. 1988; Harris and Hendrickson 1987; Houser et al. 1980; Ohara and Lieberman 1993; Pinault et al. 1995; Ralston 1983; Salt 1989; Shosaku et al. 1989).

It is known that there are at least eight metabotropic glutamate receptors (mGluRs), and these can be placed into three groups (group I: mGluR1, mGluR5; group II: mGluR2, mGluR3; group III: mGluR4, mGluR6–8) on the basis of sequence homology, agonist/antagonist pharmacology and coupling to intracellular transduction mechanisms (Nakanishi 1992; Pin and Duvoisin 1995; Watkins and Collingridge 1994). Previous work from this laboratory has shown that the GABAergic inhibition in the VB thalamus arising from the nrt can be reduced by local iontophoretic application within VB of mGluR agonists which act at receptors in groups II and III of the mGluR family (Salt and Eaton 1995a,b; Salt et al. 1996). This action is almost certainly to due to a presynaptic modulation of GABAergic transmission by these receptors (Salt and Eaton 1995a). However, some of the group II agonists which we have used previously [(2*S*, 3*S*, 4*S*)-α-(carboxycyclopropyl)-glycine e.g. (CCG-I) and (1*S*, 3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD)] are known to have appreciable activity at group I receptors. Furthermore, there is now accumulating evidence to suggest that some group I receptors may have presynaptic locations in addition to their well-known postsynaptic sites (Conquet et al. 1994; Gereau and Conn 1995; Pin and Duvoisin 1995). Thus, in order to be confident of a role for group II receptors in the modulation of sensory inhibition, it is important to investigate the actions of the more selective group II agonist 2*R*,4*R*-4-ami-

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nopyrrolidine-2,4-dicarboxylate (2*R*,4*R*-APDC; Schoepp et al. 1995), and compare its effects with those of two group I agonists, (*S*)-3,5-dihydroxyphenylglycine (DHPG; Schoepp et al. 1994) and *trans*-azetidine-2,4-dicarboxylate (*t*ADA; Favaron et al. 1993; Manahan–Vaughan et al. 1996). Using this approach, and the novel, selective group II antagonist LY307452 (Wermuth et al. 1996), we now present evidence which suggests that it is indeed group II receptors rather than group I receptors which modulate GABAergic inhibition in the VB thalamus.

Materials and methods

Experiments were carried out in adult male Wistar rats anaesthetised with urethane (1.2 g/kg i.p.), as detailed previously (Salt 1987, 1989). Extracellular recordings of single VB neurone activity were made through the central barrel (filled with 4 M NaCl) of seven-barrel glass iontophoretic microelectrodes (Salt 1987, 1989). The outer barrels were used for iontophoretic drug applications, and each contained one of the following substances, as Na^+ salts: *N*-methyl-D-aspartate (NMDA), *t*ADA, 2*R*,4*R*-APDC, (2*S*,4*S*)-2-amino-4-(4,4-diphenylbut-1-yl)-pentane-1,5-dioic acid (LY307452; all 50 mM in water, pH 8.0–8.5), DHPG (50 mM in water, pH 5.5) and 1 M NaCl (for current balancing), and Pontamine sky blue dye (2.5% in 0.5 M NaCl/0.5 M sodium acetate). All drugs were ejected iontophoretically as anions (with the exception of DHPG), and prevented from diffusing out of the pipette by a retaining current (10–20 nA) of opposite polarity to the ejection current. All drugs were obtained from Tocris, apart from 2*R*,4*R*-APDC and LY307452 (gifts from Lilly Research). Extracellular action potentials were gated and timed using a computer system which could produce peristimulus histograms (PSTHs) of single-neurone activity and which also controlled the sequences of sensory stimuli.

Sensory stimulation was carried out using electronically gated air jets (10 ms duration) which could be directed at a single facial vibrissa. In order to reveal GABAergic inhibitory processes, we used a condition-test paradigm with two air jets directed at adjacent receptive field areas, as detailed previously (Salt 1989). In such experiments it is possible to inhibit the response of a VB neurone to a test air-jet stimulus by preceding it with a conditioning stimulus to an adjacent receptive field area at an interval of 20–60 ms. The degree of inhibition can then be quantified as a percentage by the following calculation:

$$\text{Percentage inhibition} = (1 - (T_C/T) \times 100\%$$

where T is response to test stimulus (total number of action potentials evoked) and T_C is response to test stimulus when preceded by conditioning stimulus.

Using this calculation, the maximum inhibitory response which can be achieved is 100%. It is noteworthy that it was possible to evoke an inhibition of a test response with a conditioning stimulus that may in itself produce an excitatory response.

Once a protocol for revealing sensory inhibition had been established, control responses to alternate presentations of the test and condition-test stimuli (separated by 4000–5000 ms) were recorded, and these were then repeated during the concurrent application of mGluR agonists. When the effects of the agonist had been observed, the application was terminated and the sensory stimulation protocol was continued until the effects of the agonist were no longer evident.

Results

Recordings were made from 34 VB neurones, and condition-test paradigms were carried out on these with stimulus intervals of 35±1.6 ms (mean±SEM). All receptive fields were located within the vibrissal representation of the rat, and the degree of sensory inhibition observed for the population of neurones was 75±2.7%. These data for the properties of the afferent-evoked inhibition are similar to those we have presented in detail previously (Salt 1989; Salt and Eaton 1995a).

The iontophoretic application of the group II agonist 2*R*,4*R*-APDC with currents of 20–150 nA for durations of 2.5–7.5 min was found to reduce sensory inhibition on all 26 of the neurones tested in this way (Fig. 1), and this effect was statistically significant (Table 1). At

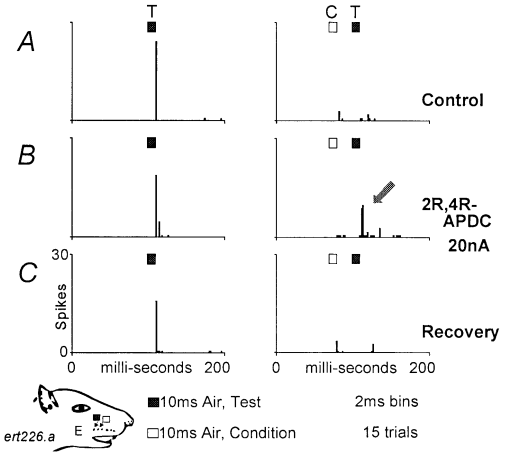

Fig. 1A–C Peristimulus time histograms of action potential spikes counted into 2-ms epochs (bins) over 15 successive trials, in response to presentation of a test stimulus alone (*left*, stimulus presented at marker *T*) or in response to presentation of a test stimulus preceded by a conditioning stimulus (*right*, marker *C*). Stimuli were directed at vibrissae as indicated on the *figurine*. **A** Responses under control conditions. **B** Responses during the continuous iontophoretic application of aminopyrrolidine-2,4-dicarboxylate *(2R,4R-APDC)*. Note that the agonist reduced the inhibition of the test stimulus by the conditioning stimulus (*arrow*). **C** Recovery from the effects of the agonist

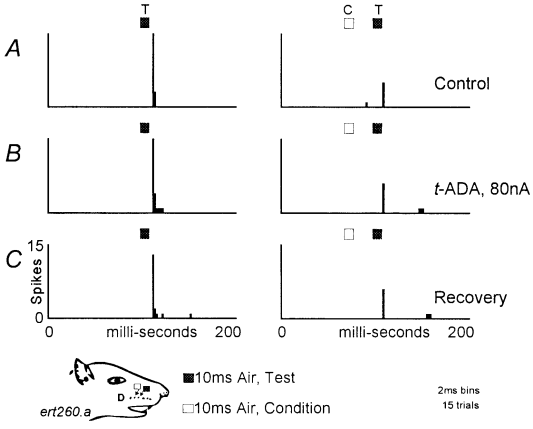

Fig. 2A–C Peristimulus time histograms of action potential spikes counted into 2-ms epochs (bins) over 15 successive trials similar to those of Fig. 1. **A** Responses under control conditions. **B** Responses during the continuous iontophoretic application of *trans*-azetidine-2,4-dicarboxylate (*t*ADA).**C** Recovery following the end of the agonist ejection

Table 1 Effects of the three agonists on sensory inhibition and responses to test stimuli alone. Data are for: sensory inhibition for each group of neurones under control conditions; sensory inhibition during coapplication of the agonist; the change in response to the test stimulus alone (percentage of preagonist control) at the same time; the iontophoretic current used for agonist application. All values are means±SEM of *n* values (*2R,4R-APDC* 2*R*,4*R*-4-aminopyrrolidine-2,4-dicarboxylate, *DHPG* (*S*)-3,5-dihydroxyphenylglycine, *tADA trans*-azetidine-2,4-dicarboxylate)

Agonist	Sensory inhibition control (%)	Sensory inhibition during agonist (%)	Test stimulus response during agonist (% control)	Iontophoretic current (nA)	*n*
2*R*,4*R*-APDC	78±3.0	25±5.3**	119±4.1**	60±6.3	26
DHPG	68±7.6	64±5.8	131±11.2**	6±2.2	9
*t*ADA	76±5.2	68±9.4	123±9.4*	105±14.8	10

** $P<0.01$; * $P<0.05$ (Wilcoxon signed-rank test)

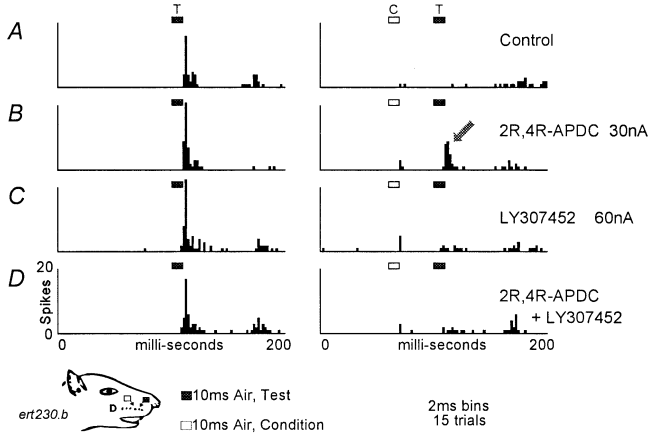

Fig. 3A–D Peristimulus time histograms of action potential spikes counted into 2-ms epochs (bins) over 15 successive trials similar to those of Fig. 1. **A** Responses under control conditions. **B** Responses during the continuous iontophoretic application of 2*R*,4*R*-APDC. Note that the agonist reduced the inhibition of the test stimulus by the conditioning stimulus (*arrow*). **C** Application of the group II antagonist LY307452 alone had little effect on responses. **D** Application of LY307452 concurrently with 2*R*,4*R*-APDC blocked the action of the agonist on sensory inhibition

the same time, responses to the test stimulus alone were slightly, but significantly, enhanced by the agonist (Table 1). In contrast, of the group I agonists, neither DHPG (1–25 nA) nor *t*ADA (40–160 nA) was found to reduce sensory inhibition (Fig. 2), although they did enhance significantly responses to the test stimuli alone (Table 1). The iontophoretic currents of these group I agonists were not increased beyond those required to produce facilitation of sensory responses, so as to avoid direct excitatory effects (Salt and Eaton 1995b).

In order to confirm that the effect of 2*R*,4*R*-APDC was indeed receptor-mediated, five of the neurones which had been studied with 2*R*,4*R*-APDC were further investigated with the group II antagonist LY307452 (Wermuth et al. 1996), applied with iontophoretic currents of 60–80 nA.

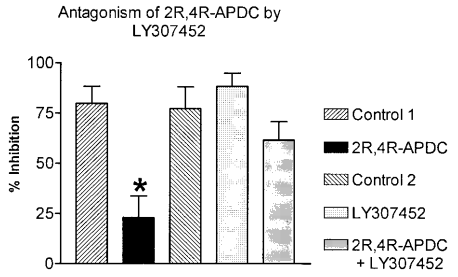

Fig. 4 Histogram showing mean (±SEM, *n*=5 neurones) values for percentage inhibition under control conditions (*Control 1*), during 2*R*,4*R*-APDC, recovery from 2*R*,4*R*-APDC (*Control 2*), during LY307452 alone, and during application of 2*R*,4*R*-APDC plus LY307452. The agonist alone produced a reduction in inhibition (*$P<0.01$, Friedman test), and this was reduced by the antagonist LY307452

This compound had no direct effect on sensory inhibition when applied alone, but it did prevent the effects of 2*R*,4*R*-APDC, as illustrated in Fig. 3 and summarised in Fig. 4.

Discussion

These results are consistent with our previous findings that agonists which have activity at group II mGluRs (e.g. 1*S*,3*R*-ACPD, CCG-I) can reduce the inhibition in VB evoked by sensory afferent stimulation (Salt and Eaton 1995a,b; Salt et al. 1996). This inhibition is known to be GABA-mediated via the input to the VB from the nrt (Salt 1989). As discussed previously (Salt and Eaton 1995a,b), it is highly likely that the effects of mGluR agonists in reducing this inhibition is via a presynaptic action to reduce the release of GABA from nrt terminals.

2*R*,4*R*-APDC has been described as a very selective agonist for mGluR2 and mGluR3 (i.e. group II), with little effect on either group I or group III mGluRs (Schoepp et al. 1995). The use of 2*R*,4*R*-APDC in this study now provides more conclusive data to show that group II mGluRs can mediate the presumed presynaptic inhibition of GABAergic inhibitory transmission. 2*R*,4*R*-APDC also resulted in a significant enhancement of responses to test air-jet stimuli alone. It is likely that this enhancement is due to a reduction in the recurrent GABAergic inhibition, which is known to occlude longer latency components to VB excitatory synaptic inputs. Indeed, work from this laboratory has shown that bicuculline can have a similar action to 2*R*,4*R*-APDC in this respect (Salt 1989).

Two agonists which have been shown to be selective for group I mGluRs, DHPG and *t*ADA, were without effect on the GABAergic inhibition, although they did enhance significantly excitatory synaptic responses to test air jet stimuli. This latter action could be attributed to a direct postsynaptic action of these agonists. Indeed, it is known that group I mGluRs have postsynaptic locations in the thalamus (Martin et al. 1992; Godwin et al. 1996 Vidnyanszky et al. 1996), and we have previously demonstrated a postsynaptic excitation of thalamic neurones me-

diated by DHPG and sensitive to group I antagonists (Salt and Eaton 1995b). However, it is of course possible that the enhancement of sensory responses by these agonists occurs via a different mechanism. Nevertheless, it is significant to note that, in the present study, neither group I agonist reduced the GABA-ergic inhibition, thus supporting the notion that group I mGluRs are not involved in the presynaptic modulation of the nrt input to VB.

Taken together with our previous data, the present findings now provide clear evidence that group II mGluRs modulate GABAergic transmission from nrt to VB together with group III mGluRs, which can be activated by L-AP4 (Salt and Eaton 1995a). In contrast there is no evidence to suggest that group I mGluRs modulate this GABAergic transmission. The modulation by group II and III receptors is consistent with the presynaptic location of some of these receptors in various brain regions with immunohistochemical techniques (Kinoshita et al. 1996; Ohishi et al. 1996; Petralia et al. 1996). However, it is noteworthy that in rat VB it has not been possible to detect an innervation of inhibitory afferent axons from nrt or their terminals by other axon terminals (Ohara and Lieberman 1993). The source of ligand which might activate presynaptic mGluRs in VB is thus, at first sight, puzzling. However, it is possible that L-glutamate or related excitatory amino acid is released non-synaptically onto these receptors, for example from astrocytes, and that this performs a local modulatory role. The factors which might govern this remain to be elucidated, but it is conceivable that the astrocytes may perform a local integrative role. An alternative or additional possibility is that astrocytes release a (non-excitatory amino acid) modulator which affects GABAergic transmission, and that this release is under the control of mGluRs. Such a scheme has been proposed for the hippocampus (Winder et al. 1996). Indeed the localisation of mGluR3 to astrocytes in many brain areas including the thalamus would lend support to such a hypothesis (Jeffery et al. 1996; Ohishi et al. 1994; Petralia et al. 1996).

The inhibitory circuit from nrt to VB is important for both spatial and temporal discrimination within the somatosensory system. The ability of mGluRs to modulate this process, possibly via a local integrative system would allow different degrees of discriminative function under possibly different physiological conditions. It has also been shown that the GABAergic inhibition is an integral part of the thalamo-cortico-thalamic loop circuit. This can function in an oscillatory mode under certain physiological/behavioural conditions, and this is critically governed by the GABAergic inhibition (Steriade and Llinas 1988; Steriade et al. 1993). It is thought that this may be central to the determination of the state of arousal of an animal, and may also be involved in processes such as absence seizures (Steriade et al. 1993). From the present data, it is possible that mGluRs may play a pivotal role in controlling this oscillatory behaviour, and thus these receptors may be of great importance in the control of arousal states and perhaps in the pathology of seizure processes.

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