Trans-ACPD and L-APB presynaptically inhibit excitatory glutamatergic transmission in the basolateral amygdala (BLA)

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(Received 12 December 1991; Revised version received 24 January 1992; Accepted 3 February 1992)

Key words: Trans-ACPD, L-APB, Synaptic transmission, Glutamate, Presynaptic, Basolateral amygdala, Intracellular recording, In vitro

Intracellular recordings were obtained from neurones of the basolateral nucleus of the amygdala (BLA) and glutamate-mediated EPSPs evoked by stimulation of the stria terminalis (ST). The conformationally restricted analogue of glutamate trans-1-aminocyclopentane-1,3-dicarboxylic acid trans-ACPD caused a dose-dependent reduction in EPSP amplitude. EC50 50 μM. This effect was mimicked by the glutamate autoreceptor agonist, 1-amino-phosphono-butryric acid (L-APB, 50 μM). Furthermore, the effects of submaximal concentrations (50 μM) of trans-ACPD and L-APB were additive. The reduction in EPSP amplitude is observed with concentrations of both drugs that have no effect on either the resting membrane potential or the input resistance of BLA neurones. In addition, these compounds can reduce EPSP amplitude but not the response to exogenous application of γ-amin-o-4-hydroxy-5-methyl-4-isoxalolpropionic acid (AMPA) suggesting activation of presynaptic receptors. These findings suggest that both trans-ACPD and L-APB act at presynaptic glutamate receptors on glutamatergic afferents to reduce excitatory transmission in the BLA.

The excitatory amino acid (EAA) glutamate is a major excitatory transmitter within the central nervous system (CNS). The response to glutamate is mediated by activation of multiple glutamate receptor subtypes. Generally, glutamate receptors can be divided into two main categories. Namely, (1) ionotropic receptors which are linked directly to ion channels, and (2) the metabotropic receptor which activates a second messenger system [24; see ref. 18]. The ionotropic receptors can be further subdivided into the N-methyl-D-aspartate (NMDA) and the kainate (KA) and 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subtypes according to their agonist profiles [3]. In contrast, the metabotropic receptor is activated by quisqualate (QUIS), ibotenate (IBO) and glutamate but not NMDA, KA or AMPA [22]. Recently, this receptor has been shown to be activated specifically by two conformationally restricted analogues of glutamate, trans-1-aminocyclopentane-1,3-dicarboxylic acid (trans-ACPD) [19] and the L-isomer of α-carboxylelropyl-glycine (L-CCG-1) [13]. A presynaptic inhibitory glutamate autoreceptor has also been described at which the L-isomer of 2-amino-phosphono-butryric acid (L-APB) appears to be the most specific agonist [4, 5, 8, 26]. Activation of this receptor causes a pathway specific decrease in excitatory postsynaptic potential (EPSP) amplitude evoked following stimulation of the lateral perforant pathway to the dentate gyrus [14], and mossy fiber input to area CA3 of the hippocampus [5, 26], as well as a decrease in glutamate release [10], and evoked field potentials [4, 11] in the hippocampus and cortex. Furthermore, L-APB causes a decrease in the amplitude of monosynaptic excitatory postsynaptic currents (EPSCs) recorded in cultured hippocampal neurones [9]. In contrast, L-APB has no effect on glutamatergic EPSPs recorded in the striatum following focal stimulation [16].

We have recently demonstrated that stimulation of the stria terminalis, the primary afferent input to the basolateral nucleus of the amygdala (BLA), evokes an EPSP that is mediated by activation of postsynaptic ionotropic NMDA and KA/AMPA receptors on BLA neurones [20]. However, evidence for the existence of functional metabotropic glutamate receptors in the BLA is limited. In a recent study Akiyama et al. [1] reported that ibotenate-stimulated polyphosphoinoside hydrolysis is increased significantly in the contralateral amygdala/pyriform cortex following kindling, indicating a possible role for metabotropic receptors in amygdala kindling.

Since trans-ACPD has been shown to cause depolarization and membrane oscillation in the septum [27] and
Fig. 1. Trans-ACP and L-APB reduce excitatory synaptic transmission in the BLA in a dose-dependent manner. A: trans-ACP and L-APB are structurally similar. B: upper traces, EPSP recorded in a BLA neuron on stimulation of the striatum before and during application of 50 μM trans-ACP. In this neuron trans-ACP reduced the EPSP amplitude by 41%. In another neuron, lower traces, the EPSP amplitude was reduced 46% following the application of 50 μM L-APB. C: neither trans-ACP (50 μM) nor L-APB (50 μM) have any effect on the current–voltage relationship generated by injection of transient (200 ms) hyperpolarising current steps of increasing amplitude in BLA neurones. D: a plot of % EPSP depression against trans-ACP concentration shows the depression to be dose-dependent with an EC₅₀ of approximately 50 μM.

also a reduction of synaptic transmission in the striatum and hippocampus [2, 7, 16], we determined the effects of trans-ACP on excitatory synaptic transmission using intracellular recording techniques from the in vitro slice of the BLA. Furthermore, as the trans-ACP-mediated reduction in synaptic transmission was similar to that previously reported for L-APB and because of the structural similarity between trans-ACP and L-APB (Fig. 1A), we also determined the effect of L-APB on synaptic transmission in the BLA.

Male Sprague–Dawley rats (110–150 g) were decapitated and the brains rapidly removed and placed in cold oxygenated artificial cerebrospinal fluid (ACSF) solution. Subsequently, the brain was hemisected and cut transversely posterior to the first branch and anterior to the last branch of the superior cerebral vein. The resulting section was glued to the chuck of a Vibroslice tissue slicer (Campden Instruments). Transverse slices of 500 μm thickness were cut and the appropriate slices placed in a beaker of oxygenated ACSF at room temperature for at least 1 h before recording. The ACSF was of the following composition (in mM): NaCl 117, KCl 4.7, CaCl₂ 2.5, MgCl₂ 1.2, NaHCO₃ 25, NaH₂PO₄ 1.2, and glucose 11. The ACSF was bubbled with 95% O₂, 5% CO₂ and had a pH of 7.4.

In the recording chamber the slice was fully submerged and maintained at 32 ± 2°C with continuously superfused ACSF. Microelectrodes were pulled from fiber-filled capillary tubing of borosilicate glass with a Flaming-Brown micropipette puller (Sutter Instruments, Model P-90). The resistance of the microelectrodes filled with 4 M potassium acetate ranged between 70 and 150 MΩ. On- and off-line data acquisition and analysis was accomplished using an Axolab 1100 interface (Axon Instruments) between an Axoclamp-2A preamplifier and a Dell 310 personal computer utilizing Pclamp 5.03 software programs (Axon Instruments). Intracellular recordings were considered acceptable if neurones exhibited overshooting action potentials and showed stable membrane potentials more negative than −60 mV in the
absence of a DC holding current. The bridge balance was carefully monitored throughout the experiments and adjusted when necessary.

Synaptic potentials were elicited orthodromically (150 µs square-wave pulses). A bipolar stainless steel electrode (Kopf Instr. SNE-200X; effective resistance 50 kΩ) was used to stimulate electrically the stria terminalis (ST). Stimulus intensities, generated by a Grass S88 stimulator, were adjusted to just subthreshold for orthodromic spike generation. EPSPs were characterized according to their amplitude and 10-90% rise time. All data are expressed as mean ± S.E.M.; in all cases, n=number of neurones. The statistical analysis used in this study was the paired Student’s t-test. Statistical significance was determined at the level of P=0.05.

All drugs were applied by superfusion in the ACSF and introduced into the recording chamber by means of a three-way stopcock. The drugs used in this study were (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA; Research Biochemicals Inc., Natick, MA); (±)-trans-1-amino-cyclopentane-1,3-dicarboxylic acid (trans-ACPD), and (±)-2-amino-4-phosphonobutyric acid (±)-APB; Toicris Neuramin, Essex, UK).

The top trace in Fig. 1B shows the ST evoked EPSP before and during superfusion of (±)-trans-ACPD (50 µM). In this neurone, trans-ACPD depressed the EPSP amplitude by 41% (mean 44 ± 7%, n=5). A similar concentration (Fig. 1B, lower trace) of ±-APB caused a 46% reduction in the amplitude of the EPSP (mean 52 ± 8%, n=7). The effect of both trans-ACPD and ±-APB were readily reversible on washout with ACSF (not shown). As shown in Fig. 1C, the current (I) and voltage (V) relationship, generated from peak voltage deflections in response to transient (200 ms) hyperpolarising current step commands of increasing intensity, were unaffected by either trans-ACPD (50 µM, closed circles) or ±-APB (50 µM, open circles). Furthermore, no significant difference was observed between the control input resistance (32.6 ± 0.3 MΩ, n=5), calculated from the slope of the I-V relationship, and the input resistance measured in the presence of either trans-ACPD (34.5 ± 1.8 MΩ, n=5) or ±-APB (35.5 ± 6.3 MΩ, n=5). Moreover, the depresant effect of trans-ACPD on the amplitude of the ST-evoked EPSP was dose-dependent (Fig. 1D, closed circles). Hence, trans-ACPD depressed the amplitude of the EPSP by 32.3 ± 7.9% (n=4) at a concentration of 20 µM whereas at 100 µM the EPSP was depressed by 68 ± 4.8% (n=5). However, at the higher concentration (100 µM) trans-ACPD caused a reduction in the input resistance of BLA neurones (control: 36.5 ± 6.0 MΩ, n=6; trans-ACPD: 34.1 ± 6.4 MΩ, n=6). This reduction in input resistance could not, however, account for the large decrease in EPSP amplitude. We have previously reported that the NMDA-receptor antagonist D-2-amino-5-phosphonovaleric acid (APV, 50 µM) can cause a small (<20%) reduction in the ST evoked EPSP in BLA neurones when recorded at −70 mV in ACSF containing physiological concentrations of Mg²⁺ [20]. In contrast, the kainate/AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 µM) can completely abolish the ST evoked EPSP. It was possible therefore, that the large reduction in EPSP amplitude observed in the presence of trans-ACPD may result from a partial blockade of postsynaptic kainate/AMPA receptors.

We examined the effect of superfusion of trans-ACPD (50 µM) on the postsynaptic response to exogenously applied AMPA (Fig. 2). In control ACSF pressure ejection of AMPA (60 ms, 1 mM) evoked a membrane depolarisation which was associated with an initial decrease in membrane input resistance (Fig. 2A, left upper trace) when recorded at −70 mV. In the same neurone stimulation of the ST evoked a typical EPSP (Fig. 2A, left bottom trace): on subsequent addition of trans-ACPD (50 µM) no reduction in the amplitude or duration of the AMPA response was observed (Fig. 2A, right upper trace) whereas the ST-evoked EPSP was reduced by 30% in this neurone (Fig. 2A, right bottom trace). Similarly, ±-APB (50 µM) had no effect on postsynaptic membrane responses evoked by AMPA (60 ms, 1 mM) application at a time when the ST-evoked EPSP was reduced in amplitude (not shown). These data support the hypothesis that both trans-ACPD and ±-APB act to reduce EPSP amplitude by an action at presynaptic receptors on glutamatergic nerve terminals. It was unclear, however, if these two compounds produced their effects by an action at the same or different receptor subtypes. We examined the effect of superfusion of a mixture of trans-ACPD (50 µM) and ±-APB (50 µM) on the amplitude of the ST-evoked EPSP and exogenously applied AMPA (Fig. 2B). The depolarising postsynaptic potential (Fig. 2B, left upper trace) evoked by pressure ejection of AMPA (60 ms, 1 mM) was unaffected by subsequent addition of trans-ACPD and ±-APB to the superfusate (Fig. 2B, right upper trace) in all neurones examined (n=3). In contrast, in the same neurone the amplitude of a ST-evoked EPSP (Fig. 2B, left bottom trace) was reduced 79% in the presence of trans-ACPD and ±-APB (mean 73.6 ± 6.3%, n=3).

These data are consistent with the view that these compounds may exert their effect on glutamate transmission by an action at different presynaptic receptor subtypes. This hypothesis is in agreement with that reported in the striatum whereby the trans-ACPD-mediated reduction in EPSP amplitude is not mimicked by ±-APB [16]. However, in the BLA these two compounds are almost equipotent in reducing EPSP amplitude at 50 µM, and hence...
may still have an additive action at the same presynaptic receptor. In addition, these data, when taken together with the reported specificity of these two compounds for glutamate receptor subtypes [5, 17, 19, 26], further suggests that these inhibitory presynaptic receptors are of the glutamatergic family. The insensitivity of striatal EPSPs to L-APB may reflect a regional distribution of metabotropic receptor subtypes. It remains to be determined if the receptors activated by these agonists correspond to the metabotropic glutamate receptor subtype. Both binding and release studies have reported that activation of the metabotropic glutamate receptor is associated with phosphotidylinositide hydrolysis and release of calcium from intracellular stores which can be antagonised by aminophosphonopropionic acid (APP) [12, 23]. In contrast, electrophysiological studies suggest that APP is a partial agonist and ineffective at blocking metabotropic receptor activation [6, 15]. This anomaly may be explained if the glutamatergic metabotropic receptor exists as a heterogeneous population as has been proposed by Tanabe et al. [25].

In conclusion, we have shown that trans-ACPD and L-APB have an additive inhibitory effect on glutamatergic transmission in the BLA. Alterations in the function of those receptors activated by these agonists may result in the enhanced glutamatergic transmission in the BLA observed following kindling [21].

This work was supported by a National Institute of Neurological Disorders and Stroke Grant NS-24643 to P.S.-G.


