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Ionotropic GABA receptors with mixed pharmacological properties of $GABA_A$ and $GABA_C$ receptors

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Abstract

Ionotropic γ-aminobutyric acid (GABA) receptors form a large family of molecular isoforms with distinct properties. We have characterized a distinct new type of GABA receptors in CA1 pyramidal cells in rat hippocampal slices. Somatic application of GABA induced currents which were partially suppressed by (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA), a specific antagonist of $GABA_C$ receptors. This sensitivity was enhanced when we evoked the currents by the $GABA_C$ receptor agonist *cis*-4-aminocrotonic acid (CACA). However, both GABA- and CACA-evoked currents were sensitive towards bicuculline and thus lack the defining feature of $GABA_C$ receptors, which are insensitive towards this antagonist. Spontaneous miniature post-synaptic currents (mIPSCs) revealed a similar pharmacological behaviour. We conclude that juvenile CA1 pyramidal cells express a fraction of ionotropic GABA receptors with mixed pharmacological properties of both, $GABA_A$ and $GABA_C$ receptors.

Keywords: Hippocampus; mIPSC; GABA receptor; Rho subunit; TPMPA; CACA

1. Introduction

Ionotropic γ-aminobutyric acid (GABA) receptors are constituted from a diverse family of homologous subunits. The underlying gene family can be sorted into several subfamilies, termed $\alpha_{(1–6)}$, $\beta_{(1–3)}$, $\gamma_{(1–3)}$, $\delta_{(1)}$, $\varepsilon_{(1)}$, $\theta_{(1)}$, $\pi_{(1)}$ (Sieghart, 1995; Whiting et al., 1999) and the family of $\rho_{(1–3)}$ subunits (Bormann, 2000). This molecular heterogeneity corresponds to functional differences between receptor isoforms which may contribute to the diversity of inhibitory synapses in the central nervous system. While the ubiquitously distributed $GABA_A$ receptors are blocked by the alkaloid bicuculline, a bicuculline-insensitive ionotropic GABA receptor has been found in the retina (Feigenspan et al., 1993; Qian and Dowling, 1993). These receptors can be distinguished from $GABA_A$ receptors by several other pharmacological and functional characteristics and have therefore been termed $GABA_C$ receptors (Drew et al., 1984). Comparison of native GABA receptors with heterologously expressed subunits revealed that $GABA_C$ receptors are composed of ρ subunits (Shimada et al., 1992; Bormann, 2000). In contrast to the heterooligomeric $GABA_A$ receptor complexes, ρ subunits form homooligomeric receptor channels when expressed in Xenopus oocytes (Shimada et al., 1992; Wang et al., 1994; Shingai et al., 1996). However, it has been shown that ρ_2 subunits can congregate with ρ_1 (Zhang et al., 1995; Enz and Cutting, 1999) or ρ_3 (Ogurusu et al., 1999). In heterologous expression systems, ρ subunits can also assemble with members of the $GABA_A$ receptor subunit family (Qian and Ripps, 1999; Ekema et al., 2002), but it is unclear whether such combinations occur in vivo.

Besides their insensitivity towards bicuculline, $GABA_C$ receptors are characterized by their high affinity to the

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natural agonist GABA, slow activation and inactivation kinetics, weak desensitisation, long channel open times and small single channel conductance (Bormann and Feigenspan, 1995). These properties are consistent with a role in tonic inhibition. Indeed, recent evidence suggests that ρ subunits are expressed by central neurons, including the hippocampus (Wegelius et al., 1998; Ogurusu et al., 1999; Strata and Cherubini, 1994; Didelon et al., 2002; Rozzo et al., 2002; Kirischuk et al., 2003). In hippocampal interneurons, Semyanov and Kullmann (2002) have described GABA responses with mixed pharmacological properties typical for both, $GABA_A$ and $GABA_C$ receptors.

Here, we describe GABA responses with mixed pharmacological properties of $GABA_A$ and $GABA_C$ receptors in hippocampal pyramidal cells in juvenile rat brain slices in vitro. While these receptors lack the defining feature of bicuculline-resistance and therefore cannot be termed $GABA_C$ receptors, they are activated by the $GABA_C$ receptor agonist *cis*-4-aminocrotonic acid (CACA) and blocked by the $GABA_C$ receptor selective antagonist (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA). Thus, principal neurons in the hippocampus express a distinct ionotropic GABA receptor subtype, which cannot unambiguously be ascribed to one of the established subgroups. The existence of such receptors emphasises doubts on whether a strict separation between $GABA_A$ and $GABA_C$ receptors is justified from a functional point of view (Bormann, 2000; Chebib and Johnston, 1999).

2. Material and methods

2.1. Slice preparation

Experiments were performed on CA1 hippocampal pyramidal neurons from slices obtained from juvenile 8- to 18-day-old Wistar rats. Within this range, we did not find any age-dependence of the results and therefore pooled the data. Procedures were approved by the state government of Berlin and are in accordance with NIH guidelines. In brief, the animals were decapitated after cervical dislocation, the brain was removed and immersed into ice-cold (~4 °C) sucrose solution which contained (in mM): sucrose 75, NaCl 87, KCl 2.5, NaH_2PO_4 1.25, $NaHCO_3$ 26, $MgCl_2$ 7, $CaCl_2$ 0.5, glucose 25, saturated with 95% O_2–5% CO_2. Horizontal slices of the hippocampus (250 μm in thickness) were prepared using a Vibratome (Campden, Leicestershire, UK). Slices were incubated in sucrose solution at 34 °C for 30 min, followed by storage at room temperature (20–22 °C).

2.2. Whole-cell recordings

For measurements, slices were transferred to a submerged-type chamber and were superfused with artificial cerebrospinal fluid (ACSF) at 34 °C containing: NaCl 124, KCl 3, NaH_2PO_4 1.25, $NaHCO_3$ 26, $MgSO_2$ 1.8, $CaCl_2$ 1.6, glucose 10, saturated with 95% O_2–5% CO_2, pH 7.3. Whole-cell patch clamp recordings were achieved from visually identified pyramidal cells using DIC video microscopy (Axioscope, Zeiss, Germany) and an EPC-7 amplifier (List Medical, Darmstadt, Germany). Pipette resistance was 2–5 MΩ leading to series resistances between 5 and 25 MΩ, which were regularly controlled throughout the experiment. Intracellular solutions were based either on K-gluconate (140 mM) or CsCl (140 mM). In addition, solutions contained (in mM): $MgCl_2$ 2, HEPES 10, EGTA 5, MgATP 2, pH 7.25 adjusted with either KOH or CsOH. Drugs were applied either by bath perfusion or by brief pressure pulses to the soma from a closely located micropipette (10 ms, 2 mbar). The experimental protocol for drug delivery was standardised in order to maintain repeatable and comparable results (distance from application pipette to somatic membrane ~10 μm; HEPES (10 mM)-buffered ACSF in pressure pipette; each response averaged from three separate applications at 6-s intervals). Effects of antagonists on GABA- or CACA-evoked currents were tested after pre-equilibration of the bath with the antagonist for ~3 min and with the antagonist included in the pressure pipette solution (see Results). Drugs were obtained from Sigma (Deisenhofen, Germany). All recordings (currents evoked by pressure-application of drugs as well as spontaneous miniature post-synaptic currents (mIPSCs)) were performed in the continuous presence of 0.5 μM tetrodotoxin, 30 μM DL-2-amino-5-phosphonovaleric acid (APV), 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 1 μM (2*S*)-3-[[(1*S*)-1-(3,4-dichlorophenyl) ethyl]amino-2-hydroxypropyl](phenylmethyl)phosphinic acid (CGP55845). The latter two substances were dissolved from ×1000 stock solutions in dimethylsulfoxide, which was present at equal concentration in all applied solutions throughout the experiments. mIPSCs were recorded at a holding potential of −60 mV. Currents were amplified (10 mV/pA), filtered at 3 kHz and stored on PC using TIDA (HEKA, Lambrecht, Germany) software (sampling rate 8 kHz).

2.3. Data analysis

GABA- and CACA-evoked currents were analysed within the TIDA program. Detection and analysis of mIPSCs was performed offline by an automated event detection algorithm using Spike 2 (Cambridge electronics Design, Cambridge, UK). The detection trigger level was set at 1.8× standard deviation of event-free baseline noise (typically ≤5 pA). Reliability of this detection criterion was assessed by comparison with hand-evaluated individual traces.

Statistical tests were performed with the non-parametric Wilcoxon tests ($P<0.05$ regarded as significant and indicated by *; $P<0.01$ indicated by **). Data are presented as mean±S.E.M.

3. Results

3.1. Antagonist action on GABA-evoked currents

In a first series of experiments, we applied the natural agonist GABA to the somatic membrane of CA1 pyramidal cells by brief pressure pulses. At −20 mV holding potential with K-gluconate-based intracellular solution, 100 μM GABA elicited outwardly directed currents of 1244±129 pA amplitude (n=19). When GABA was applied together with increasing concentrations of the competitive $GABA_A$ receptor antagonist bicuculline, currents became smaller and were almost abolished at 100 μM bicuculline (<1% of control amplitude; Fig. 1A,B). Fitting the data with a logistic equation yielded an apparent IC_{50} of 4.3 μM.

While these data are well compatible with the behaviour of $GABA_A$ receptors, subsequent experiments revealed features which are more reminiscent of $GABA_C$ receptors. GABA-evoked currents were also reduced by the $GABA_C$ receptor antagonist TPMPA (60 μM; Fig. 2A). In a first series of experiments, GABA was applied from the pressure pipette before and after addition of TPMPA to the bath solution. In an alternative protocol, GABA was applied together with TPMPA from the pressure pipette and the bath was pre-equilibrated with the antagonist for ~3 min before application of the drug. The latter design does reflect the antagonist action of TPMPA more precisely because it excludes that the antagonist is being washed out by the stream of agonist-containing solution from the application-pipette. It does not allow, however, for a direct comparison within one cell. Both approaches yielded a similar suppression of GABA-evoked currents by 27±16% (n=10; bath application of TPMPA) or 22±15% (n=4; TPMPA in bath and pipette; Fig. 2A). The effect of bath-applied TPMPA could not be washed out within 10 min (n=4, data not shown).

The current remaining under 60 μM TPMPA could be almost abolished by bath application of bicuculline (30 M), leaving only 1% of the basal GABA response at 100 μM

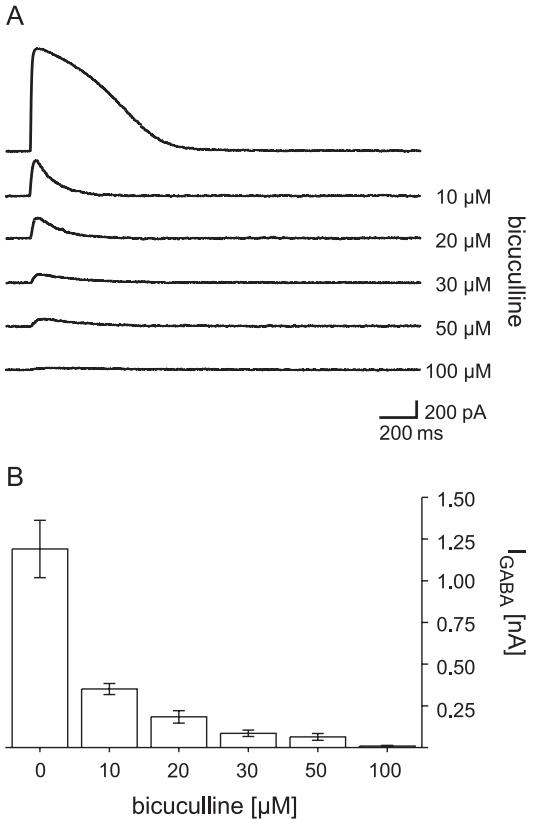

Fig. 1. Block of GABA-evoked currents by bicuculline. (A) Example traces showing current responses of a CA1 pyramidal cell to GABA (100 μM; pressure-application to the soma; holding potential −20 mV) in the presence of increasing concentrations of bicuculline. The antagonist was applied with the bath solution. Note almost complete block of current at 100 μM bicuculline. (B) Average current amplitudes without and with increasing concentrations of bicuculline. In these experiments, bicuculline was present in both, pressure pipette and bath (one data point from each cell, five to seven cells for each concentration).

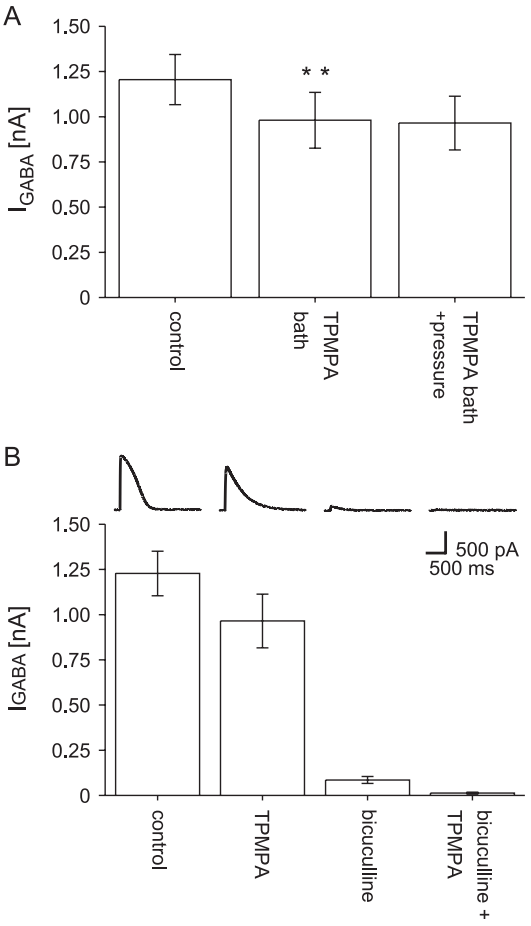

Fig. 2. Effects of $GABA_C$ and $GABA_A$ receptor antagonists on GABA-evoked currents. (A) Mean current amplitude under control conditions (n=19), after addition of 60 μM TPMPA to the bath (n=10, P<0.05) and in cells with TPMPA in both, pipette and bath (n=4). Currents are reduced by ~25% in both conditions. (B) Comparison of individual and combined antagonist effects. Example traces mounted on top of respective bar. All experiments were performed with drugs in bath and pipette. Bicuculline was applied at 30 μM, TPMPA at 60 μM.

(n=4; Fig. 2B). This combined effect of bicuculline and TPMPA indicates that the underlying receptor population exhibits a mixed sensitivity towards both antagonists.

We also tested the effects of TPMPA on GABA-evoked currents after iontophoretic application of the agonist (1 mM, pH 4.0, adjusted with HCl). Addition of TPMPA to the bath solution yielded results similar to the pressure-application protocol (suppression by 17±42%; n=12). However, GABA receptors have been shown to be differentially sensitive to external proton concentration, depending on their subunit composition (Krishek et al., 1996). In order to avoid any bias for certain molecular subtypes, we therefore continued the measurements with pressure-applied agonists.

3.2. Agonist effects on GABA receptors

In order to further distinguish GABA receptor subtypes on CA1 pyramidal cells, we applied the $GABA_C$ receptor agonist CACA and compared the effects to the non-selective natural ligand GABA. Indeed, pressure-application of CACA (1 mM) reproducibly induced outward currents at three different holding potentials with an extrapolated reversal potential of −64 mV (n=5). This was similar to the reversal potential of GABA (100 μM)-evoked currents (−63 mV, n=19) and is consistent with the activation of predominantly chloride-selective ion channels (Fig. 3A). Despite the high concentrations of both agonists used, currents evoked by CACA were generally smaller than those induced by GABA.

The fraction of receptors activated by CACA was more sensitive to the $GABA_C$ receptor antagonist TPMPA than the previously tested GABA-evoked currents. At 60 μM, the antagonist suppressed the CACA-evoked current by 65±18% (Fig. 3B; n=6; compare to 22% block of GABA-induced currents). In these experiments, TPMPA was contained in both, the bath solution as well as the application pipette in order to avoid washout effects (see above). Again, the effects were not readily reversible within about 10 min of wash. Thus, CACA selectively activates chloride-selective ion channels with enhanced sensitivity towards TPMPA.

If CACA-evoked currents in CA1 pyramidal cells were mediated by $GABA_C$ receptors, they should be insensitive towards bicuculline. Fig. 3C shows that this was not the case: currents were almost abolished (<1% remaining amplitude; n=5) by 30 μM bicuculline. Thus, CACA-evoked currents in CA1 pyramidal cells are highly sensitive to the $GABA_A$ receptor blocker bicuculline and therefore are not mediated by $GABA_C$ receptors.

3.3. Synaptic currents

We wondered whether TPMPA-sensitive ionotropic GABA receptors may contribute to post-synaptic GABAergic currents. In order to exclude potential network or

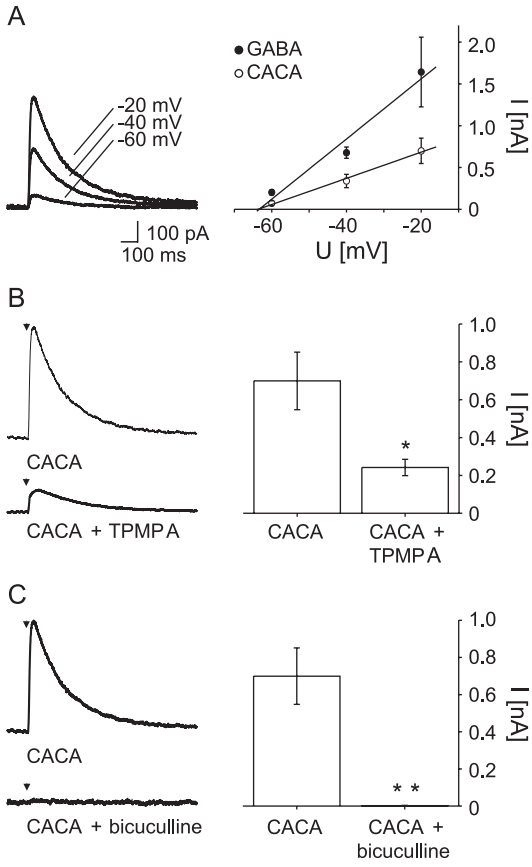

Fig. 3. Properties of currents evoked by CACA (1 mM). (A) Outward currents elicited at different holding potentials reveal a reversal potential typical for Cl^-. Note similar potential dependence of GABA- and CACA-evoked currents. (B) Currents evoked by CACA are highly sensitive towards TPMPA. Bar diagram shows reduction by ~65% (n=6, P<0.05). (C) CACA-evoked currents are almost completely blocked by 30 μM bicuculline (n=5, P<0.01).

presynaptic effects, these experiments were performed in the presence of tetrodotoxin, thus measuring mIPSCs. When TPMPA was added to the bath-solution, amplitudes of mIPSCs were apparently reduced (Fig. 4A). Quantification of mIPSC amplitudes revealed a leftward shift of the cumulative amplitude distribution curve and reduced the mean mIPSC amplitude by 29% (Fig. 4B,C; n=11, P<0.01). The kinetics of mIPSCs was analysed by averaging 10 events from each cell and calculating mono- and bi-exponential decay time constants as well as the half-width. Mean values of all kinetic parameters were slightly increased by TPMPA, but only the half-width changed significantly (8.69±1.05 vs. 11.35±1.14 ms, n=11 cells, P<0.05). Thus, kinetic changes were subtle and, altogether, indicate a rather fast decay kinetics of TPMPA-sensitive GABA receptors (Fig. 4E). As apparent from Fig. 4A, the frequency of mIPSCs was reduced in the presence of TPMPA (mean frequency ~80% of control, data not shown). It should be noted, however, that small mIPSCs may have been further reduced by TPMPA and subsequently escaped

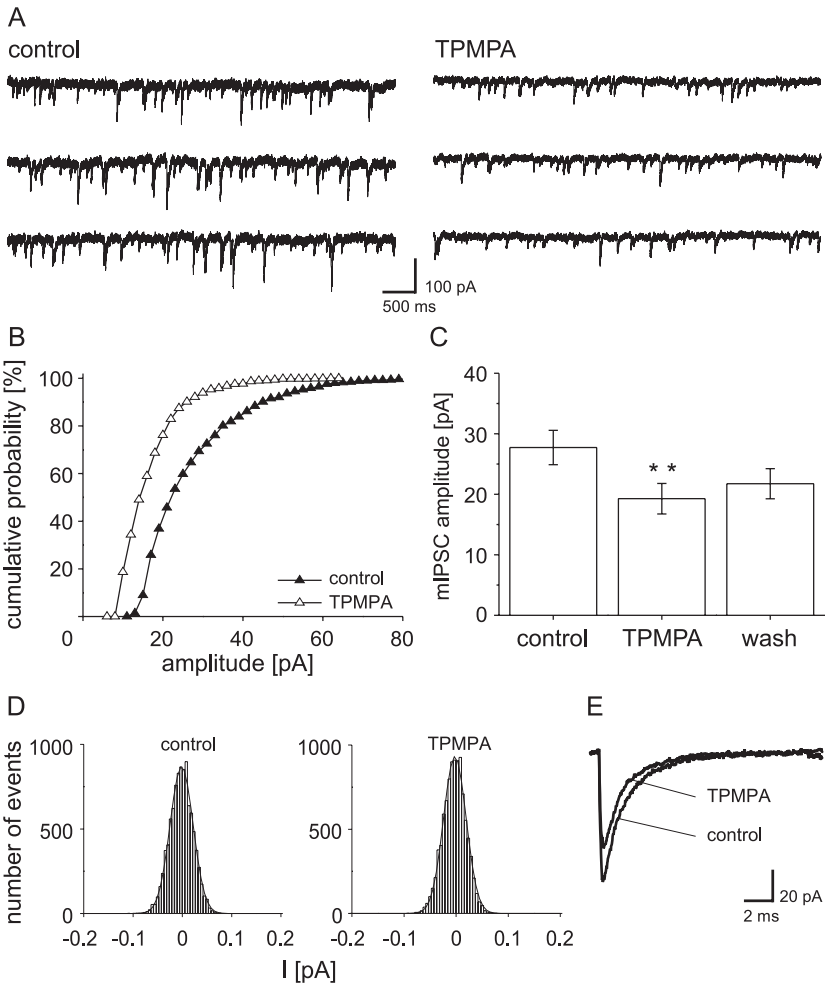

Fig. 4. Miniature inhibitory post-synaptic currents (mIPSC) are suppressed by TPMPA (60 μM). (A) Example of original recording in the absence (left) and presence (right) of TPMPA. Note apparent decrease in event frequency. (B) Cumulative mIPSC amplitude histogram sampled from one cells shows clear leftward shift by TPMPA. (C) Mean amplitude reduction by TPMPA (n=11 cells, P<0.01). (D) Representative all-point-amplitude histograms of 1.2 s of event-free recordings under control conditions and in the presence of TPMPA. The antagonist induced no significant reduction in baseline noise. (E) Kinetics of mIPSCs (example traces averaged from 10 events). Panel shows superimposed curves of mIPSCs under control conditions and after TPMPA application (see text).

detection. Therefore, the apparent reduction of mIPSCs frequency has to be interpreted with great caution.

3.4. Noise

Finally, we assessed the involvement of TPMPA-sensitive GABA receptors in tonic inhibition by measuring baseline noise before and after addition of the antagonist. All-point-amplitude-histograms were constructed from 1.2 s of mIPSC-free current traces and were fitted with the Gaussian equation (Fig. 4D). The half-width of these baseline noise histograms did not, however, change in the presence of TPMPA (0.060±0.006 pA under control condition vs. 0.055±0.005 pA after application of TPMPA; n=11, P>0.25) indicating that TPMPA-sensitive GABA receptors do not add significantly to baseline noise. In two cells, we added the $GABA_A$ receptor antagonist bicuculline together with TPMPA. This reduced the baseline noise by 43% and 26%, respectively, indicating that there is indeed some tonic GABAergic inhibition in these cells.

4. Discussion

The present study shows that rat CA1 pyramidal cells express an unusual type of chloride-selective ionotropic GABA receptors. The channels are sensitive to bicuculline, consistent with known properties of $GABA_A$ receptors. They are, however, selectively activated by CACA and blocked by TPMPA, as typical for bicuculline-insensitive $GABA_C$ receptors.

4.1. Pharmacological profile

$GABA_C$ receptors have been defined as ionotropic GABA receptors which are distinguished from $GABA_A$

receptors by their unique pharmacological properties, most notably their insensitivity towards block by the plant alkaloid bicuculline (Johnston et al., 1975; Drew et al., 1984). Additional pharmacological features of $GABA_C$ receptors include their activation by the GABA analogue CACA (Johnston et al., 1975), and their sensitivity towards the specifically designed antagonist TPMPA (Ragozzino et al., 1996; for review, see Bormann and Feigenspan, 1995). Our findings are partially compatible with these typical properties of $GABA_C$ receptors, but lack the crucial and defining criterion of bicuculline resistance.

GABA-evoked currents in CA1 pyramidal cells were clearly reduced by TPMPA. This hybrid of isoguvacine and 3-APMPA has been designed to retain its affinity for $GABA_C$ receptors but not to interact with $GABA_A$ or $GABA_B$ receptors, respectively. $GABA_C$ receptors composed of ρ_1 subunits are blocked by TPMPA with an apparent K_d of ~2 μM as compared to ~320 μM for $GABA_A$ receptors. In addition, the substance has weak agonist activity at $GABA_B$ receptors (Ragozzino et al., 1996), which was excluded as a confounding factor in our experiments by the antagonist CGP55845. In physiological experiments, TPMPA has been used at concentrations between 10 and 300 μM in order to selectively suppress the $GABA_C$ receptor-mediated component (e.g., Kirischuk et al., 2003; Semyanov and Kullmann, 2002; Boller and Schmidt, 2001; Shen and Slaughter, 2001; Rozzo et al., 2002). At 60 μM, as used in the present study, effects on $GABA_A$ receptors are minimal (Ragozzino et al., 1996; Johnston, 2002; Wall, 2001). Therefore, we conclude that a fraction of about 30% of the ionotropic GABA receptors on CA1 pyramidal somata display sensitivity towards TPMPA, demonstrating a pharmacological quality typical for $GABA_C$ receptors.

While GABA-induced currents were only moderately suppressed by TPMPA, we could strongly enhance the fraction of TPMPA-sensitive channels by application of the $GABA_C$ receptor agonist CACA, which revealed robust outward currents in CA1 pyramidal cells. Besides $GABA_C$ receptors, CACA can also activate certain $GABA_A$ receptors at high concentrations (Kusama et al., 1993). However, such currents are not sensitive towards TPMPA, as has been shown for α_6-containing $GABA_A$ receptors in cerebellar granule cells (Wall, 2001). In our study, CACA-evoked currents were clearly more sensitive towards TPMPA than currents elicited by the common agonist GABA. Thus, CACA selectively activated a receptor population different from typical $GABA_A$ receptors. This finding does also exclude that the CACA-evoked currents were carried by typical $GABA_A$ receptors due to a contamination with the common agonist *trans*-4-aminocrotonic acid (TACA), similar to the findings by Semyanov and Kullmann (2002). It is also not likely that the enhanced percentual block of CACA-evoked currents by TPMPA is due to their lower amplitude: GABA-evoked currents consistently showed 20–30% suppression by TPMPA, irrespective of their amplitude. This was also true for the small (~300 pA) currents evoked by GABA-iontophoresis. Thus, CACA-evoked currents are enriched in a pharmacologically distinct receptor fraction proving that different molecular subtypes of GABA receptors are present at somatic CA1 pyramidal cell membranes, one of which shows certain features of $GABA_C$ receptors.

In contrast to the $GABA_C$ receptor-like profile demonstrated above, all GABA-activated currents in hippocampal CA1 neurons showed a defining property of $GABA_A$ receptors: both the GABA- and the CACA-evoked currents were clearly sensitive towards bicuculline, reaching an almost complete block of GABA-evoked currents at 100 μM. Thus, the GABA receptors under study can not be ascribed to the $GABA_C$ receptor subtype. This statement must be extended to the fraction of CACA-activated, highly TPMPA-sensitive currents which were almost completely abolished by 30 μM bicuculline and are, therefore, $GABA_A$ receptor-mediated. Even when we evoked currents by a lower concentration of CACA (100 μM) they remained sensitive towards bicuculline, although any fraction of "true" $GABA_C$ receptors should have been even more enlarged under these conditions (data not shown). Altogether, our pharmacological data hint towards the presence of ionotropic GABA receptors in CA1 pyramidal cells which share properties of $GABA_C$ receptors while they formally belong to the $GABA_A$ receptor family.

4.2. Molecular composition and distribution

At the molecular level, $GABA_C$ receptors have been identified by their composition from ρ_{1-3} subunits (Cutting et al., 1992; Polenzani et al., 1991; Woodward et al., 1992, 1993; Ogurusu and Shingai, 1996). These subunits are most prominently expressed in the retina and other nuclei of the visual system (mammals: Feigenspan et al., 1993; Enz et al., 1995, 1996; avian: Albrecht and Darlison, 1995; amphibian: Lukasiewicz et al., 1994; fish: Qian and Dowling, 1993) but have also been identified in various other regions of the brain (Wegelius et al., 1998; Boue-Grabot et al., 1998; Enz and Cutting, 1999; Ogurusu et al., 1999). In the hippocampus, there is evidence for the expression of different ρ subunits in the CA1 and CA3 pyramidal layers (Wegelius et al., 1998; Ogurusu et al., 1999; Boue-Grabot et al., 1998). Recent evidence suggests that both ρ_1 and ρ_2 subunits are present in the pyramidal cell layers, but show decreasing levels towards adulthood with persisting high expression in interneurons (Strata and Cherubini, 1994; Didelon et al., 2002; Rozzo et al., 2002). However, the occurrence of ρ subunits in the hippocampus does not account for the bicuculline-sensitivity of the respective receptors.

This raises the question of whether co-assemblies of ρ subunits with conventional $GABA_A$ receptor subunits might

underlie the channels with mixed properties of both, $GABA_A$ and $GABA_C$ receptors. While ρ subunits share up to 78% amino acid sequence identity among each other, they are also closely related to subunits of the $GABA_A$ receptors with which they have 30–38% sequence homology (Cutting et al., 1992; Bailey et al., 1999). It has indeed been shown that ρ_1 can co-assemble with the $GABA_A$ receptor γ_2 subunit (Qian and Ripps, 1999; Ekema et al., 2002) as well as with the glycine receptor α_1 and α_2 receptor subunits in vitro (Pan et al., 2000). The kinetic and pharmacological properties of these receptors closely resemble native $GABA_C$ receptors. At present, it is unclear whether combinations of $GABA_A$ and $GABA_C$ receptor subunits occur in vivo and whether such combinations can show a functional profile similar to the receptors described here. Such combinations may also underlie the recently discovered atypical ionotropic GABA receptors in hippocampal CA1 interneurons (Semyanov and Kullmann, 2002). Similar to our present data, these authors found currents with mixed pharmacological properties of $GABA_A$ and $GABA_C$ receptors. Our findings and the data by Semyanov and Kullmann might also be explained by an interaction between closely co-localized $GABA_C$ and $GABA_A$ receptors. Alternatively, there might be pure combinations of $GABA_A$ receptor subunits, which yield the observed atypical pharmacological profile (see, e.g., Wall, 2001, for CACA-activated $GABA_A$ receptors). The hippocampus contains multiple $GABA_A$ receptor subunits and knowledge about the functional properties of their possible combinations is incomplete.

4.3. Functional significance

What is the functional significance of the GABA receptor type identified in this study? Despite their pharmacological properties, $GABA_C$ receptors are characterized by sustained activation with little desensitisation and by their high agonist affinity. This makes them ideal candidates for tonic background inhibition at extrasynaptic sites, similar to $GABA_A$ receptor subtypes with high agonist affinity in hippocampal and cerebellar granule cells (Brickley et al., 2001; Stell and Mody, 2002). Our recordings of mIPSCs revealed, however, that the fraction of subsynaptically localized TPMPA-sensitive GABA receptors is similar to the amount found by pressure application. Conversely, they did not obviously contribute to the "background" noise measured in mIPSC-free sections of our recordings. This indicates that the atypical GABA receptors are not selectively positioned at extrasynaptic sites and do not serve a major function in tonic background inhibition (similar to the atypical receptors described by Semyanov and Kullmann, 2002).

In summary, our data reveal an atypical ionotropic GABA receptor in CA1 pyramidal cells with subsynaptic localisation and mixed pharmacological properties of $GABA_A$ and $GABA_C$ receptors. The specific functions of these receptors as well as their molecular composition remain to be elucidated. Future work at the molecular level has to reveal whether native GABA receptors can be composed of $GABA_A$ and $GABA_C$ receptor subunits, which would make the strict distinction between both subtypes obsolete.

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