2-Aminoethyl Diphenylborinate Blocks GABAA-Receptor-Mediated Currents in Rat Medial Preoptic Neurons

Michael Druzin* and Staffan Johansson

Department of Integrative Medical Biology Umeå University, SE-901 87 Umeå, Sweden
* Corresponding e-mail: michael.druzin@umu.se

Abstract. The effect of 2-aminoethyl diphenylborinate (2-APB), a commonly used drug to modulate inositol-1,4,5-triphosphate (IP3) receptors and transient receptor potential (TRP) channels, on GABAA receptor-mediated currents was studied in neurons from the medial preoptic nucleus (MPN) of rat. 2-APB gradually and reversibly reduced the currents evoked by GABA but had no effect on the currents evoked by glycine. The blocking effect was not mediated by alterations in intracellular calcium concentration and showed a concentration dependence with half maximal effect at ~50 µM 2-APB, for currents evoked by 100 µM, as well as by 1.0 mM GABA, suggesting that 2-APB is not competing with GABA for its binding site at the GABAA receptor. Thus, the present study describes a novel pharmacological property of 2-APB as a non-competitive blocker of GABAA receptors and calls for caution in the interpretation of the results where 2-APB is used to affect IP3 receptors or TRP channels.

Introduction

GABA (γ-aminobutyric acid) is the most widespread inhibitory neurotransmitter in the mammalian brain. Released from GABA-ergic neurons GABA binds the GABAA receptors, which predominantly mediate rapid synaptic inhibition (for review see Farrant & Nusser, 2005). The GABAA receptor consists of five subunits forming a transmembrane channel permeable to chloride ions and and bicarbonate, to a smaller degree. An influx of negatively charged chloride ions through this channel upon activation of the GABAA receptor results in hyperpolarization of the cellular membrane and thus neuronal inhibition (Rudolph et al., 2001; Sieghart & Sperk, 2002). GABAA receptors contain a number of well-established modulatory sites, which are targets for several pharmacologically and clinically important drugs, such as benzodiazepines, barbiturates, steroids, anti-epileptics and anesthetics (Macdonald & Olsen, 1994). In addition, some of the nonsteroidal anti-inflammatory drugs, fenamates containing a diphenyl group, modulate GABAA receptors (Woodward et al., 1994). 2-aminoethyl diphenylborinate (2-APB), a molecule sharing structural similarities with fenamates, is a well-known pharmacological agent widely used in neuroscience. This membrane permeable molecule is commonly believed to selectively modulate the intracellular calcium concentration, $[Ca^{2+}]i$, acting at several targets in neurons (Maruyama et al., 1997). Thus, 2-APB blocks inositol 1,4,5-triphosphate (IP3) receptors, which are Ca^{2+} permeable ion channels residing in the membrane of the endoplasmic reticulum (Dobrydneva & Blackmore, 2001; Tao & Harris, 2007). 2-APB is also known to modulate several types of the transient receptor potential (TRP) channels, which permit Ca^{2+} flux across the cell membrane (Clapham, 2003; Xu et al., 2005). Therefore, observed effects of 2-APB on various neuronal functions, such as neurotransmission and synaptic plasticity, are usually interpreted as consequences of 2-APB-evoked changes in $[Ca^{2+}]i$ (Miyakawa et al., 2001).

The present study addresses the effect of 2-APB on GABAA receptor function in neurons from the rat medial preoptic nucleus (MPN). On the basis of perforated-patch clamp recordings, a novel and unexpected effect of 2-APB as a potent blocker of neuronal GABAA receptors is described. Clarification of the blocking effect of 2-APB on GABAA receptors may provide a basis for the development of new pharmacological agents and call for thorough reinterpretation of previous findings concerning the effect of 2-APB on neurons and their functions.

Methods

Ethical approval

Ethical approval of the procedures described was given by the regional ethics committee for animal research ("Umeå djurförsöksetiska nämnd", approval no. A9-14).

Cell preparation

Male Sprague-Dawley rats were used for the experiments. The neurons studied were taken from young rats weighing 60 - 100 g. The animals were killed by decapitation without anaesthetics. The brain was quickly removed and placed in preoxygenated ice-cold incubation solution. The meninges were removed, a block of tissue including the anterior hypothalamus and preoptic area was cut out, and slices, 300 µm thick, were cut using a vibroslicer (Campden instruments, Leicestershire, UK). The prepared slices were incubated for 1.5 - 2 hours in incubation solution (see below) at 27 - 28°C. After the incubation period, the slices were transferred to a plastic dish, and neurons were mechanically dissociated by approaching the tip of a vibrating glass rod towards the MPN (cf Karlsson et al., 1997a). No enzymes were used. Dissociated cells were allowed to settle at the bottom of the dish for 30 minutes.

Electrophysiological recordings

Membrane currents were recorded using both the conventional whole-cell and amphotericin-B-perforated-
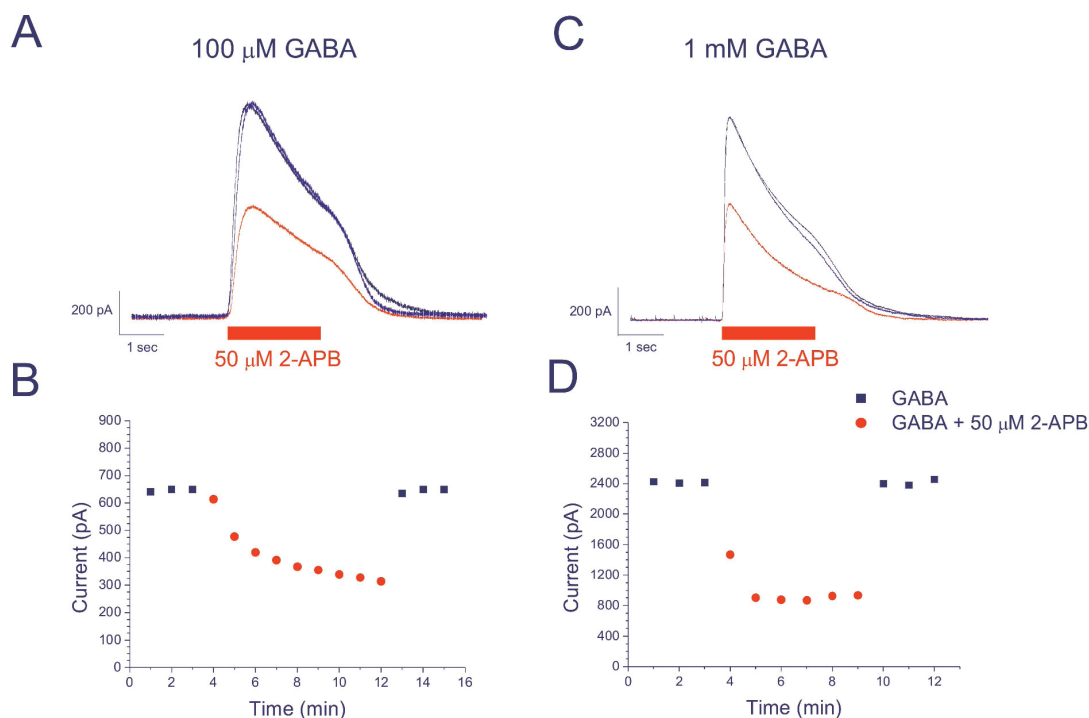
Figure 1. Blocking effect of 50 μM 2-APB on the currents evoked by repetitive application of GABA. (A) Raw currents recorded from an MPN neuron in response to 100 μM GABA only (black), in the presence of 50 μM 2-APB at the 5th consecutive application of 100 μM GABA (red) and after wash-out of 2-APB (blue). (B) Time course of the effect of 2-APB on peak current amplitude, for the cell in A (black: control; red: 2-APB). (C) Raw currents recorded from an MPN neuron in response to 1.0 mM GABA only (black), in the presence of 50 μM 2-APB at the 5th consecutive application of 1.0 mM GABA (red) and after wash-out of 2-APB (blue). (D) Time course of the effect of 2-APB on peak current amplitude, for the cell in C (black: control; red: 2-APB). Currents were recorded at -34 mV.

patch-clamp technique in voltage-clamp conditions at a steady membrane potential of -34 mV, where the driving force for GABA-evoked currents is significant, but the activation of steady voltage-gated currents does not impart significant extra noise. Borosilicate glass pipettes (Harvard apparatus, Kent, UK) were used. The pipette tips were filled by immersion in intracellular solution and subsequent back-filling with intracellular solution. When immersed in standard extracellular solution, the pipette resistance was 2.5 – 3.5 MΩ. Series resistance compensation was not used due to non-exponential components in capacitive transients. Slow changes in series resistance less than about 20 % were accepted. The liquid-junction potential was about 14 mV and has been subtracted in all potential values given. The recordings were made using an Axopatch 200A amplifier, a Digidata 1200 interface and the pClamp software (all from Axon instruments, Foster city, USA). The extracellular solution, without or with GABA and 2-APB, was applied by a gravity-fed fast perfusion system with a four- or eight-barrelled pipette positioned 100 – 200 μm from the cell under study. All experiments were carried out at room temperature, 21 - 23 °C.

Chemicals and solutions

The incubation solution contained (in mM): NaCl 150, KCl 5.0, CaCl2 2.0, HEPES 10, glucose 10, Tris-base 4.9. This solution was oxygenated. The standard extracellular solution contained (in mM): NaCl 137, KCl 5.0, CaCl2

1.0, MgCl2 1.2, HEPES 10, glucose 10, glycine (3.0 μM), tetrodotoxin (2.0 μM). pH was adjusted to 7.4 with NaOH. In some experiments EGTA (10 mM) was added to buffer Ca^{2+}. The intracellular solution, used for amphotericin-B-perforated-patch recordings, contained (in mM): Cs-gluconate 140, NaCl 3.0, MgCl2 1.2, EGTA 1.0, HEPES 10. pH was adjusted to 7.2 with CsOH. Amphotericin-B (Sigma-Aldrich) was dissolved in dimethylsulphoxide (DMSO; 1.2 mg in 20 μl DMSO). The final concentration of DMSO in the intracellular solution was 0.28 %. The intracellular solution, used for conventional whole-cell recordings, contained (in mM): Cs-gluconate 125, Na-acetate 3.0, NaCl 6, CaCl2 0.9, Mg-ATP 5.0, Na2-GTP 0.4, BAPTA 3, HEPES 10, pH was adjusted to 7.2 with CsOH. 2-APB (Sigma-Aldrich) was first dissolved in 99.5 % ethanol, for preparation of solutions containing a final ethanol concentration of 0.2 %. Glycine (Sigma-Aldrich) and GABA (Sigma-Aldrich) were directly dissolved in extracellular solution. Liquid-junction potentials were calculated using the Clampex software (versions 9 & 10; Molecular Devices, CA, USA) and have been subtracted in all potentials given. Free Ca^{2+} concentrations in the presence of Ca^{2+} chelators EGTA and BAPTA were calculated using the web-based open-access calculator Maxchelator (http://web.stanford.edu/~cpatton/maxc.html).

Data analysis

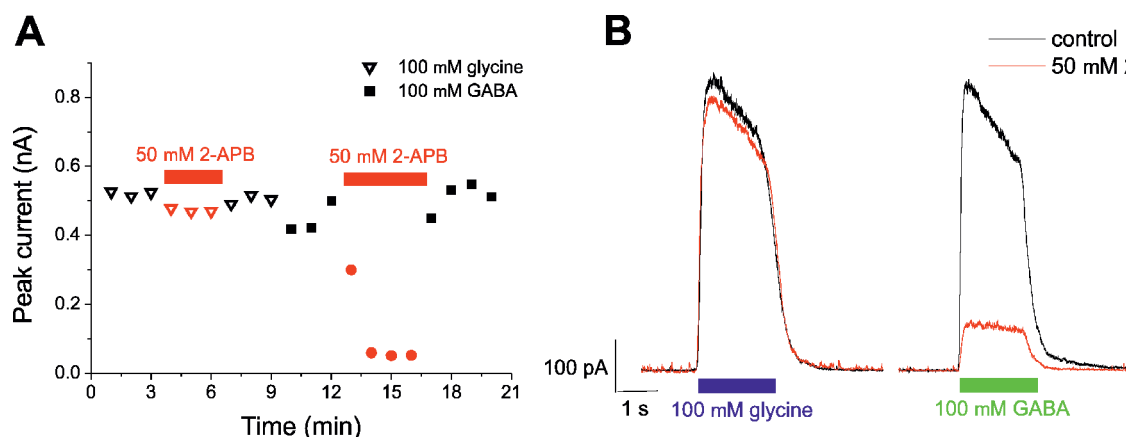All analysis, including curve fitting, was performed using the pCLAMP software (Axon Instruments) and

Figure 2. 2-APB selectively blocks GABA-evoked currents. (A) Raw currents recorded from an MPN neuron in response to 100 μM glycine (left) and 100 μM GABA (right) in control conditions without 2-APB (black curves) and in the presence of 50 μM 2-APB (3rd consecutive application; red curves). (B) Time course of the effect of 2-APB on the amplitude of the peak current evoked by 100 μM glycine (open triangles) and 100 μM GABA (filled squares) for the cell in A (black: control; red: 2-APB). Glycine-evoked currents were reduced to 97 ± 6 %. Recordings were made as in Fig. 1.

the Origin software (Microcal Software). The current amplitude was measured semi-manually, using cursors. The concentration-response curve was generated by fitting the logistic (Hill) equation:

$$I = I_{max} / (1 + (EC_{50}/C)^n) \qquad \text{(Equation 1)}$$

to the data, where I is current with subscript "max" denoting maximum current, C denoting concentration of 2-APB, EC_{50} the half-saturating concentration and n the "Hill slope". The two-sample Kolmogorov-Smirnov test was used to statistically evaluate the obtained results. Data are presented as mean ± S.E.M.

Results

2-APB blocks GABA-evoked currents

External application of GABA onto dissociated MPN neurons gives rise to membrane currents through GABA$_A$ receptors (Karlsson et al., 1997b; Karlsson et al., 2011). Under the present conditions, with the membrane potential clamped at -34 mV, 100 μM GABA delivered directly via the perfusion pipette elicited an outward current with a peak amplitude of 891 ± 168 pA (n = 16). After the peak, the GABA-evoked current decayed during 2.0 seconds of GABA application (Fig. 1A), reflecting partial desensitization of GABA$_A$ receptors in combination with intracellular accumulation of Cl⁻ that reduces the driving force for Cl⁻ (Karlsson et al., 2011). To study the effect of 2-APB, we used repetitive application of GABA with time intervals of 1.0 minute to allow recovery of GABA$_A$ receptors from desensitization as well as restoration of intracellular chloride concentration via membrane chloride transporters and passive diffusion into the recording pipette. In control conditions, the peak current remained stable at repetitive GABA application (black dots in Fig. 1B, D).

After switching to a solution containing 50 μM 2-APB, the peak amplitude of GABA-evoked current was

gradually reduced with time, to a near steady level (Fig. 1A, B). On average, 50 μM 2-APB decreased the peak amplitude of the currents evoked by 100 μM GABA to 57 ± 8 % during the first 5 GABA applications from the start of incubation in 2-APB. The blocking effect of 2-APB was fully reversible within 1 - 2 minutes of washing-out 2-APB.

To clarify the selectivity of the blocking effect of 2-APB, the repetitive applications of 100 μM glycine were made using the same protocol as with GABA applications. In agreement with previous observations (Karlsson et al., 1997b; Karlsson et al., 2011), glycine
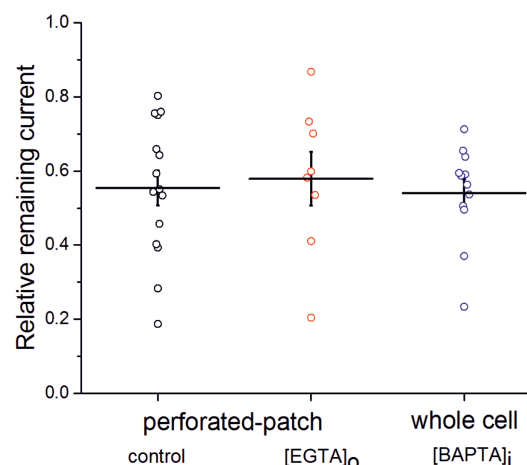

Figure 3. Buffering of intra- or extracellular Ca²⁺ did not prevent the blocking action of 2-APB on GABA-evoked currents. Neither chelating of extracellular Ca²⁺ with 10 mM EGTA (n = 8; red open circles) nor chelating of intracellular Ca²⁺ with 3.0 mM BAPTA (n = 12; blue open circles) affected the 2-APB-induced reduction of GABA-evoked currents as compared to control situation (n = 16; black open circles) with 1.0 mM extracellular Ca²⁺ and unbuffered intracellular Ca²⁺. Currents were recorded and analyzed as in Fig. 1. Recordings in control solution and in EGTA-containing solution were made using the amphotericin-B-perforated patch technique; recordings with intracellular BAPTA were made using the conventional whole-cell method with BAPTA delivered to the cell through the recording pipette.
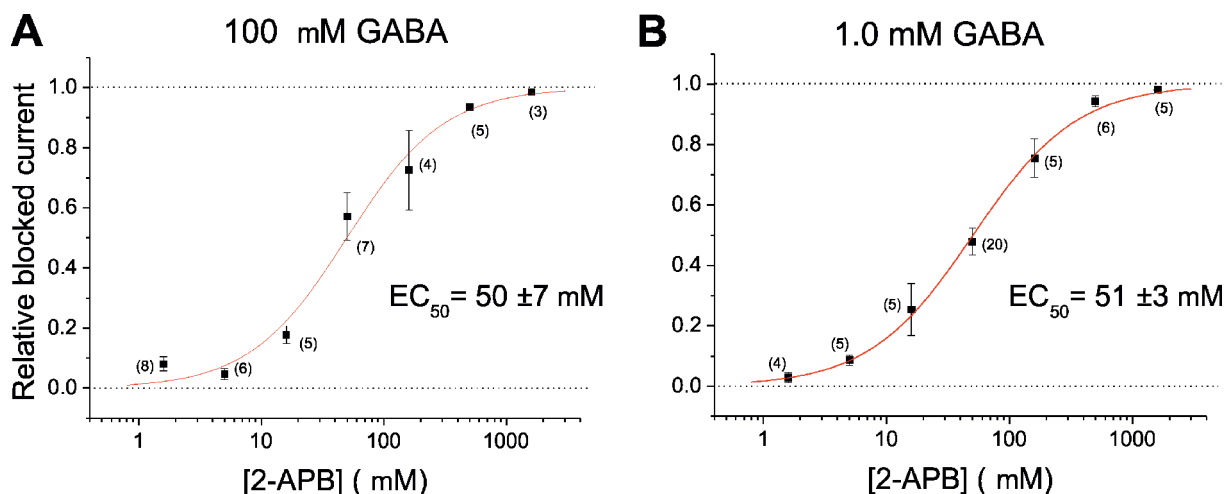
Figure 4. Concentration-response curve for the 2-APB-induced block of the current evoked by GABA application. (A) Amplitude of the blocked portion of the peak current evoked by 100 μM GABA in the presence of 50 μM 2-APB normalized to the peak amplitude evoked by 100 μM GABA alone. Data represent averages from 3 - 8 cells (shown in brackets). The bars denote S.E.M. The smooth line is described by Equation 1 with an EC50 value of 50 μM and a Hill coefficient of 1.02. (B) Amplitude of the blocked portion of the peak current evoked by 1.0 mM GABA in the presence of 50 μM 2-APB normalized to the peak amplitude evoked by 1.0 mM GABA alone. Data represent averages from 4 - 20 cells (shown in brackets). The bars denote S.E.M. The smooth line is described by Equation 1 with an EC50 value of 51 μM and a Hill coefficient of 1.01. GABA-evoked currents were recorded at -34 mV.

applications produced outward currents with kinetics similar to the currents evoked by GABA. However, in contrast to GABA-evoked currents, the glycine-evoked currents were not affected in the 3 cells tested (Fig. 2A, B), suggesting that 2-APB is targeting GABAA receptors but not glycine receptors.

As 2-APB is known to modulate $[Ca^{2+}]i$, the observed blocking effect of 2-APB on GABA-evoked currents could be mediated by changes in $[Ca^{2+}]i$ since GABAA receptors are highly regulated by intracellular Ca^{2+}-dependent mechanisms (Stelzer, 1992). To rule out possible involvement of 2-APB-senstitve Ca^{2+} channels in the plasma membrane, extracellular Ca^{2+} was buffered with 10 mM EGTA, expected to result in ~70 nM free Ca^{2+}. When the effect of 2-APB was tested in EGTA-containing extracellular solution, the degree of block of GABA-evoked current did not significantly differ (p = 0.99) from that observed in our previous experiments in control solution with 1.0 mM free Ca^{2+} (Fig. 3). Further, to test possible contribution of 2-APB action on intracellular Ca^{2+} stores, conventional whole-cell recordings were made, using an intracellular (pipette) solution with 3.0 mM BAPTA, expected to reduce free $[Ca^{2+}]i$ to ~35 nM. Similarly to the experiments with EGTA, buffering of $[Ca^{2+}]i$ by BAPTA did not prevent the blocking action of 2-APB on GABA-evoked currents: No significant difference (p = 0.65) from control experiments was observed (Fig. 3). Thus, the blocking effect of 2-APB on GABA-evoked currents is not mediated by Ca^{2+}.

The relation between 2-APB concentration and the degree of current block at 100 μM GABA was investigated with 2-APB concentrations ranging from 500 nM to 1.6 mM. The data obtained were fitted by equation 1 (see Methods), giving a half-maximal block (EC50) at 50 μM 2-APB and a Hill coefficient of 1.02 (Fig. 4A).

2-APB is not a competitive blocker of GABAA receptors

To clarify the mechanism of 2-APB block, we investigated whether 2-APB is competing with GABA at the binding site on the GABAA receptor. For this, we repeated the experiments as shown above, but with the GABA concentration increased to 1.0 mM. If the blocking effect of 2-APB is competitive, then the 2-APB concentration for half-maximal block (EC50) should increase with GABA concentration. However, for currents evoked by 1.0 mM GABA, the EC50 for 2-APB-induced block was 51 μM and Hill coefficient of 1.01, thus similar to that for 100 μM GABA (Fig. 4B), suggesting that 2-APB does not compete with GABA at the binding site of the GABAA receptor.

2-APB is a possible open-channel blocker of GABAA receptors

To further clarify the mechanism of 2-APB action at the GABAA receptors, the time course of the 2-APB-induced block was compared for 100 μM GABA and 1.0 mM GABA. In a previous study, 100 μM GABA has been shown to evoke a maximum response, likely saturating GABAA receptors expressed in MPN neurons (Karlsson et al., 2011). Nevertheless, with the present experimental paradigm, 1.0 mM GABA evoked a larger current (1040 ± 207 pA, n = 21) than did 100 μM GABA (891 ± 168 pA, n = 16), suggesting that 100 μM GABA may not activate all available GABAA receptors during a single application. We hypothesized that 2-APB requires open GABAA receptors for its blocking effect. If this is the case, the 2-APB-induced block at repetitive GABA application should occur at a slower rate with 100 μM GABA than with 1.0 mM GABA, since a higher number of applications should be needed to activate all GABAA receptors and

make them available for 2-APB block. Indeed, the results were consistent with this hypothesis, showing that the block of the current evoked by 100 μM GABA occurred with a time constant of 2.9 ± 0.9 GABA applications (n = 6), whereas the block of current evoked by 1.0 mM GABA was much faster, with a time constant of only 0.9 ± 0.6 GABA applications (n = 14; Fig. 1B, D).

Discussion

The results presented here demonstrate, to our knowledge for the first time, that 2-APB blocks GABAA receptor-mediated currents. Unlike some of the known blockers, such as picrotoxin, which may target both the GABAA receptors and glycine receptors (Zhorov & Bregestovski, 2000), the blocking action of 2-APB is relatively selective for GABAA receptors. The finding that 2-APB blocks GABAA receptors is surprising since 2-APB has long been used in neuroscience as a selective modulator of cellular Ca^{2+} signaling, acting at targets which are very different from the GABAA receptor. Thus, 2-APB modulates Ca^{2+} release from intracellular Ca^{2+} stores into the cytoplasm via IP3-gated Ca^{2+} channels (IP3 receptors) as well as Ca^{2+} entry through various types of Ca^{2+} permeable TRP channels in the cellular membrane (Dobrydneva & Blackmore, 2001; Clapham, 2003; Xu et al., 2005; Tao & Harris, 2007). It is also known that GABAA receptor function can be modulated by $[Ca^{2+}]i$. Low $[Ca^{2+}]i$ was reported to increase GABAA receptor function, while an appreciable increase in $[Ca^{2+}]i$ (>1 μM) was found to decrease GABAA receptor function via phosphorylation-dephosphorylation mechanisms (Aguayo et al., 1998; Isokawa, 1998; Kittler & Moss, 2003; Mozrzymas & Cherubini, 1998). The Ca^{2+} sensitivity of the GABAA receptor may be considered likely as mediating the blocking effect of 2-APB, which is known to affect $[Ca^{2+}]i$ (see above). However, the results presented show that neither buffering extra- nor intracellular Ca^{2+} affected the blocking action of 2-APB, suggesting a Ca^{2+}-independent mechanism of GABAA receptor block by 2-APB.

The EC50 value for 2-APB-induced block of GABAA receptors in the present study is well within the effective range of 2-APB when affecting IP3 receptors and TRP channels (Dobrydneva & Blackmore, 2001; Clapham, 2003; Xu et al., 2005; Tao & Harris, 2007). For this reason, the findings here may provide a basis for possible reinterpretation of previous studies of 2-APB action on neuronal function and signaling. Thus, in cases where GABAA-receptor activation cannot be excluded, altered GABAA-receptor mediated changes in Cl- and HCO3- flux and membrane potential may have contributed to the observed effects of 2-APB.

GABAA receptors are known to be modulated by a number of substances whose actions reflect the central role of GABA-ergic neurotransmission in the control of CNS excitability (Davies, 2003, Maguire & Mody, 2009; Mihic, 1999). Some of these substances such as benzodiazepines, barbiturates and steroids potentiate GABAA receptor-mediated currents whereas plant convulsants such as picrotoxin and bicuculline inhibit GABAA receptors (Macdonald & Olsen, 1994). Drugs affect GABAA receptor-mediated currents via action on channel conductance, opening probability and/or open duration. Classical GABAA receptor blockers like picrotoxin and bicuculline act via different mechanisms of block, as well as at different sites at the GABAA receptor. Thus, bicuculline produces a competitive block of GABA-evoked currents by competing with GABA for its binding site at the GABAA receptor, whereas picrotoxin exerts a noncompetitive inhibition via a site distinct from that for GABA, most likely physically blocking the channel (DeLorey & Olsen, 1992). The present findings suggest that the mechanism of 2-APB-induced block of GABAA receptor is different from that of bicuculline (Akaike et al., 1985) since 2-APB is not competing for the GABA binding site. Further, the GABA concentration affects the rate of 2-APB-induced block suggesting that activation of the GABAA receptor is required for the blocking effect. Thus, 2-APB is likely to act as an open-channel blocker similarly to tetraethyl ammonium (TEA), a quaternary ammonium ion, which was first described by Armstrong (1971) as an open channel blocker of voltage-gated potassium channels.

The results provide a possible clue to the mechanisms of GABAA receptor modulation by fenamates, drugs which are structurally similar to 2-APB. Although fenamates are widely used clinically as nonsteroidal anti-inflammatory drugs, they have recently been shown to also act at GABAA receptors (Uusi-Oukari & Maksay, 2006). The here suggested property of 2-APB to block only activated GABAA receptors could be very useful in studies of GABAA receptor-mediated neurotransmission where activity-dependent changes in synaptic efficacy are often observed. Possibly, understanding the blocking mechanism may also help to develop new pharmaceuticals for the treatment of pathological conditions involving GABA-ergic dysfunction, such as epilepsy, schizophrenia and chronic pain (Ben-Ari, 2006; Guidotti et al., 2005; Zeilhofer et al., 2012).

Acknowledgements

References

AKAIKE N., HATTORI K., OOMURA Y. & CARPENTER D.O. (1985): Bicuculline and picrotoxin block gamma-aminobutyric acid-gated Cl- conductance by different mechanisms. Experientia 41, 70-71.

ARMSTRONG C.M. (1971): Interaction of tetraethyl ammonium ion derivatives with the potassium channels of giant axons. Journal of General Physiology 58, 413-437.

AGUAYO L.G., ESPINOZA F., KUNOS G. & SATIN L.S. (1998): Effects of intracellular calcium on GABAA receptors in mouse cortical neurons. Pflügers Archives

435, 382-387.

BEN-ARI Y. (2006): Seizures beget seizures: the quest for GABA as a key player. Critical Reviews in Neurobiology 18, 135-144.

CLAPHAM D.E. (2003): TRP channels as cellular sensors. Nature 426, 517-524.

DAVIES M. (2003): The role of GABAA receptors in mediating the effects of alcohol in the central nervous system. Journal of Psychiatry and Neuroscience. 28, 263–274.

DOBRYDNEVA Y. & BLACKMORE P. (2001): 2-Aminoethoxydiphenyl borate directly inhibits store-operated calcium entry channels in human platelets. Molecular Pharmacology 60, 541-552.

DELOREY T.M. & OLSEN R.W. (1992): γ–Aminobutyric acidA receptor structure and function. Journal of Biological Chemistry 267, 16747-16750.

FARRANT M. & NUSSER Z. (2005): Variations on an inhibitory theme: phasic and tonic activation of GABAA receptors. Nature Neuroscience Reviews 6, 215-229.

GUIDOTTI A., AUTA J., DAVIS J.M., DONG E., GRAYSON D.R., VELDIC M., ZHANG X. & COSTA E. (2005): GABAergic dysfunction in schizophrenia: new treatment strategies on the horizon. Psychopharmacology (Berl) 180, 191-205.

ISOKAWA M. (1998): Modulation of GABAA receptor-mediated inhibition by postsynaptic calcium in epileptic hippocampal neurons. Brain Research 810, 241-250.

KARLSSON U., SUNDGREN A.K., NÄSSTRÖM J. & JOHANSSON S. (1997a): Glutamate-evoked currents in acutely dissociated neurons from the rat medial preoptic nucleus. Brain Research 759, 270-276.

KARLSSON U., HAAGE D. & JOHANSSON S. (1997b): Currents evoked by GABA and glycine in acutely dissociated neurons from the rat medial preoptic nucleus. Brain Research 770, 256-260.

KARLSSON U., DRUZIN M. & JOHANSSON S. (2011): Cl$^-$ concentration changes and desensitization of GABAA and glycine receptors. Journal of General Physiology 138, 609-626.

KITTLER J.T. & MOSS S.J. (2003): Modulation of GABAA receptor activity by phosphorylation and receptor trafficking: implications for the efficacy of synaptic inhibition. Current Opinion in Neurobiology 13, 341-347.

MACDONALD R.M. & OLSEN R.W. (1994): GABAA receptor channels. Annual Review of Neuroscience 17, 569-602.

MAGUIRE J. & MODY I. (2009): Steroid hormone fluctuations and GABA(A)R plasticity. Psychoneuroendocrinology 34, 84-90.

MARUYAMA T., KANAJI T., NAKADE S., KANNO T. & MIKOSHIBA K. (1997): 2APB, 2-aminoethoxydiphenyl borate, a membrane-penetrable modulator of Ins(1,4,5) P3-induced Ca^{2+} release. Journal of Biochemistry 122, 498-505.

MIHIC S.J. (1999): Acute effects of ethanol on GABAA and glycine receptor function. Neurochemistry International 35, 115-123.

MIYAKAWA T., MIZUSHIMA A., HIROSE K., YAMAZAWA T., BEZPROZVANNY I., KUROSAKI T. & IINO M. (2001): Ca^{2+}-sensor region of IP3 receptor controls intracellular Ca^{2+} signalling EMBO Journal 20, 1674–1680.

MOZRZYMAS J.W. & CHERUBINI E. (1998): Changes in intracellular calcium concentration affect desensitization of GABAA receptors in acutely dissociated P2-P6 rat hippocampal neurons. Journal of Neurophysiology 79, 1321-1328.

RUDOLPH U., CRESTANI F. & MOHLER H. (2001): GABAA receptor subtypes: dissecting their pharmacological functions. Trends in Pharmacological Sciences 22, 188–194.

SIEGHART W. & SPERK G. (2002): Subunit composition, distribution and function of GABAA receptor subtypes. Current Topics in Medical Chemistry 2, 795–816.

STELZER A. (1992): Intracellular regulation of GABAA-receptor function. In Ion: Channels, (Ed Narahashi T.), pp. 83-136, Plenum, New York.

TAO L. & HARRIS A.L. (2007): 2-aminoethoxydiphenyl borate directly inhibits channels composed of connexin26 and/or connexin32. Molecular Pharmacology 71, 570-579.

XU S.Z., ZENG F., BOULAY G., GRIMM C., HARTENECK C. & BEECH D.J. (2005): Block of TRPC5 channels by 2-aminoethoxydiphenyl borate: a differential, extracellular and voltage-dependent effect. British Journal of Pharmacology 145, 405-414.

WOODWARD R.M., POLENZANI L. & MILEDI R. (1994): Effects of fenamates and other nonsteroidal anti-inflammatory drugs on rat brain GABAA receptors expressed in Xenopus oocytes. Journal of pharmacology and experimental therapeutics 268, 806-817.

UUSI-OUKARI M. & MAKSAY G. (2007): Allosteric modulation of [3H] EBOB binding GABAA receptors by diflunisal analogues. Neurochemistry International 49, 676-682.

ZEILHOFER H.U., WILDNER H. & YÉVENES G.E. (2012): Fast synaptic inhibition in spinal sensory processing and pain control. Physiological Reviews 92, 193-235.

ZHOROV B.S. & BREGESTOVSKI P.D. (2000): Chloride Channels of Glycine and GABA Receptors with Blockers: Monte Carlo Minimization and Structure-Activity Relationships. Biophysical Journal 12, 1786–1803.