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Benzodiazepine modulation of recombinant $\alpha 1\beta 3\gamma 2$ $GABA_A$ receptor function efficacy determination using the Cytosensor microphysiometer

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Abstract

γ-Aminobutyric acid (GABA) dose dependently increased extracellular acidification rate in Ltk^- cells stably expressing human recombinant $\alpha 1\beta 3\gamma 2$ $GABA_A$ receptors but had no effect in non-transfected controls. Cells seeded at 1×10^5 cells/cup, with 4–5 days induction, had basal acidification rates of 105 ± 2 μVs^{-1} at 37°C (mean ± standard error of mean, $n = 37$). GABA responses had a characteristic time-course with an initial alkalinisation followed by a peak of acidification, which was optimized by increasing agonist exposure from 15 s to 25–30 s. The maximum concentration of GABA tested (100 μM) produced a 40 ± 2% increase over basal acidification rate ($n = 3$), with an EC_{50} of 15.5 μM and a Hill slope of 1.5. Responses were specifically antagonized by bicuculline and could be modulated by benzodiazepine ligands with varying efficacies. Full benzodiazepine agonists flunitrazepam (1 μM) and zolpidem (10 μM) significantly potentiated the response to 10 μM GABA by 124 ± 15% ($n = 7$) and 117 ± 23% ($n = 3$), respectively. The partial agonist bretazenil (100 nM) produced a 45 ± 13% ($n = 3$) potentiation whilst the inverse agonist DMCM (10 μM) (methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate) inhibited the response to 20 μM GABA by 53 ± 5%. The microphysiometer offers an alternative functional measure for $GABA_A$ receptors with the sensitivity to measure subtle modulatory effects of benzodiazepine site ligands and to determine their relative efficacy.

Keywords: $GABA_A$; Modulation; Benzodiazepine; Efficacy; Microphysiometer

1. Introduction

γ-Aminobutyric acid type A ($GABA_A$) receptors are GABA-gated chloride channels with multiple subunits (α1-6, β1-3, γ1-3, δ and ε) which coassemble as pentamers to form a family of receptor subtypes which are differentially expressed in mammalian brain. $GABA_A$ receptors mediate inhibitory neurotransmission in the central nervous system and belong to the ligand-gated ion channel receptor family. Ion channel opening can be modulated via a variety of allosteric sites on the receptor (Sieghart, 1992) and these modulatory sites are the target for a number of therapeutic agents, such as benzodiazepines, barbiturates, steroids and anaesthetics. Affinity and efficacy at the benzodiazepine site are influenced by α and γ (McKernan et al., 1995; Buhr et al., 1996; Wingrove et al., 1997) but not β subunits (Hadingham et al., 1993). The benzodiazepine binding site is formed at the interface of an α subunit (1–6) and a γ subunit (1–3) with contributions from both governing modulation and benzodiazepine pharmacology (Smith and Olsen, 1995; Wingrove et al., 1997).

A variety of techniques have been used to measure $GABA_A$ receptor function of which the most sensitive, but labour-intensive, is electrophysiology. Other techniques used have included ^{36}chloride flux (Kardos, 1993) and allosteric modulation of radioligand binding as a correlate of function (Sieghart, 1995). The Cytosensor microphysiometer (Molecular Devices, CA) uses silicon-based potentiometric sensors to measure subtle changes in extracellular acidification rates (Parce et al., 1989), resulting from changes in cell energy demand during receptor and ion channel activation, or from alterations in Na^+/H^+ exchange across the membrane and regulation of intracellular pH, and may offer an alternative functional measure for $GABA_A$ receptors. Cells acidify their extracellular environment as a consequence of any stimulus which increases the rate of production of the acidic products of energy metabolism (McConnell et al., 1992). Although these changes in extracellular acidification rate are downstream

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from the primary stimulus and signal transduction events, the ubiquitous nature of such changes in cellular metabolism has allowed many different receptor systems and signalling pathways to be studied. Activation of a wide variety of receptors has been characterised using microphysiometry (McConnell et al., 1992) including G-protein coupled receptors (Neve et al., 1992), growth factor receptors (Pitchford et al., 1995), and ligand-gated ion channel receptors, specifically ionotropic glutamate receptors (Raley-Susman et al., 1992; Trafton et al., 1996).

A preliminary report was recently published describing the first study of the application of microphysiometry to the investigation of human recombinant $GABA_A$ receptor function in stable cell lines (Smith et al., 1996). The present paper further describes the characteristics of recombinant $GABA_A$ receptor responses measured using microphysiometry and describes the development of an assay to measure stably expressed $GABA_A$ $\alpha1\beta3\gamma2$ receptor function, including the determination of relative efficacy for compounds which modulate ion channel activity via the benzodiazepine site.

2. Materials and methods

2.1. Tissue culture and cell preparation

Mouse Ltk^- fibroblast cells expressing receptors with subunit composition $\alpha1\beta3\gamma2$ were maintained in stock trays in Dulbecco's modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 1 mg/ml geneticin. Stocks were split 1:8 into fresh trays once a week. Prior to seeding, cells (passage 5 to 25) were removed from stock plates with 0.05% trypsin/0.53 mM EDTA solution (Gibco) and resuspended in DMEM supplemented with 10% fetal calf serum, 100 units/ml penicillin and 100 μg/ml streptomycin but without geneticin. Cells were subsequently seeded into 12 mm transwell inserts (Costar) at a variety of densities between 5×10^4 and 2×10^5 cells per cup in 0.5 ml of this same medium and a further 1 ml medium was applied to the surrounding well. The following day receptor expression, which is under the control of a dexamethasone-sensitive promoter, was induced with this supplemented medium containing 1 μM dexamethasone and the cells were maintained for 3–6 days at 37°C in an incubator. Serum, at 10%, was present throughout the induction period until the start of the experiment.

2.2. Optimisation of conditions

Cells were seeded at 3 densities (5×10^4, 1×10^5, 2×10^5) and visually examined to assess degree of confluency before testing, aiming for a single layer of cells at 80–90% confluency. Experiments were conducted at 37°C with sensor chambers set to this temperature and debubblers set to 43°C. A sham switch was routinely performed at the beginning of each experiment to rule out any switching artifacts. Time courses were performed with GABA to determine optimal agonist exposure times, since $GABA_A$ receptors undergo rapid desensitization (Mierlak and Farb, 1988). Because of day to day variations in response latency, a limited time-course of 15, 25 and 30 s of continuous flow exposure to GABA prior to the pump-off period was carried out at the start of every experiment. The period of washout necessary for recovery between successive doses of GABA was tested by starting with a 30 min period and progressively reducing this until a decline in subsequent response size was observed.

2.3. Measurement of extracellular acidification rate and responses to GABA

Cell cups were loaded into microphysiometer sensor chambers and perfused at a rate of 100 μl/min with low buffered running medium (DMEM containing 2 mM L-glutamine, 4500 mg/l D-glucose and without bicarbonate (Sigma); supplemented with 44 mM NaCl, pH 7.4). Cells were then left to attain stable baseline acidification rates at 37°C for approximately 1 h, whilst being constantly perfused with fresh medium. Total pump cycle duration was 1 min and the pumps were on for the first 38 s. Flow was then halted between 38 and 60 s, allowing the medium bathing the cells to acidify according to cellular metabolic rate, and the rate of acidification was determined over a period of 15 s (between 43 and 58 s) whilst the pumps were off. At 1 min the pumps switched back on again to wash out the chamber.

GABA additions were superimposed on this pump cycle and GABA was applied at the following times into the cycle: at 23 s for 15 s of agonist perfusion before flow was halted at 38 s for rate determination, at 13 s for a 25 s agonist exposure, and at 8 s for a 30 s agonist exposure. To compensate for the different affinities of the benzodiazepine site ligands tested, compounds were evaluated at a fixed concentration relative to their binding affinities ($1000 \times K_i$). When examining the effect of modulatory compounds, a series of switches was made. The first was into medium containing the modulatory agent at the required concentration and this was allowed to perfuse for a period of 8 min. The second switch was into GABA in the presence of the modulator for whatever optimal period had been determined from the earlier time-course study, typically 25 or 30 s (see Results), and finally, a switch back into modulator alone before washing out with normal medium. Test compounds were diluted into running medium from stocks in dimethyl sulfoxide (DMSO) and final solvent concentrations were kept below 0.1%. In each experiment 2 chambers out of every 8 contained non-transfected Ltk^- cells as controls. Cells were stable up to 8 h in vitro, the maximum duration of experiments.

2.4. Data handling and analysis

The majority of data is presented in the form of derived acidification rate data plotted against time, with values $>100\%$ representing increases in acidification rate (illustrated in Fig. 1). Acidification rates were measured for $n=8$ chambers per experiment and were initially recorded in μVs^{-1}. In each experiment, 2 chambers contained non-transfected parental cells as controls. To control for variations in basal acidification rate between populations of cells in different chambers, responses were subsequently normalized with respect to the baseline point immediately before agonist addition and are expressed as % increase over basal acidification rate (100%), taking the mean of the 6 responding chambers as the result for an individual experiment. Results from individual experiments were then averaged and are presented as overall mean $\pm$ standard error of mean. For benzodiazepine modulators, % potentiation of the response to GABA was also calculated: (GABA + modulator) − (GABA alone)/(GABA alone) $\times$ 100. An example of raw data from which the rate data is derived is illustrated in Fig. 3a. The rate of extracellular acidification was measured between 43 and 58 s during the period when the perfusion pumps were turned off, as the slope of a linear least-squares fit to the relation of pH vs. time (i.e., the slope of each downstroke in Fig. 3a) (Parce et al., 1989). The mean dose response curve shown in Fig. 2 was fitted by non-linear least squares regression analysis using RS1 (BBN Research Systems, Cambridge, MA, USA) and the curves in Fig. 3b were plotted using Cricket Graph (version 1.3.2).

3. Results

3.1. Optimisation of cell density and days of induction

A number of different cell densities were examined after an induction period of 4 days to determine future optimal seeding density. At 5×10^4 cells per transwell cup, cell density was low with a patchy distribution leading to variable experimental responses (data not shown). At 1×10^5 cells/cup, visual examination revealed a single layer of cells at 80–90% confluence and robust acidification responses were recorded in response to 30 μM GABA. At the highest density of cells examined, 2×10^5 cells/cup, cells were beginning to overgrow and form patches of more than monolayer thickness and, in addition, responses to 30 μM GABA were poorer. Initial acidification rates for cells optimally seeded at 1×10^5 cells/cup with 4 days of growth and induction varied between 60 and 160 μVs^{-1} after system calibration and equilibration of individual chamber responses. The mean basal acidification rate of these cells was 105 ± 2 μVs^{-1} (mean and standard error of mean, $n=37$). Of the varying number of days of induction examined, 4 to 5 days of induction was

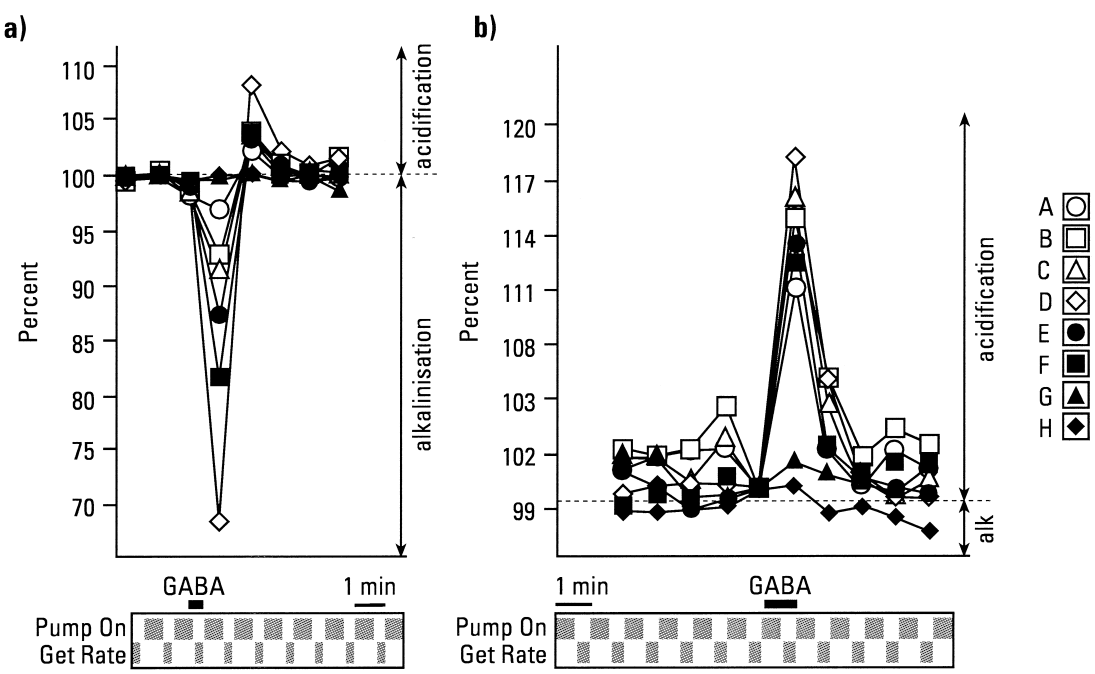


Fig. 1. Time-dependency of different components of the response to 10 μM GABA illustrating the need for optimisation of agonist dosing schedules. Results, from a representative experiment, are plotted as calculated acidification rate vs. time for 8 parallel chambers, with the response from each chamber normalized with respect to the baseline point immediately before agonist addition. Values $>100\%$ represent increases in acidification rate. Panel (a) shows the rapid alkalinisation optimally observed using a short agonist exposure of 15 s and (b) shows the more pronounced acidification optimally observed at 30 s. Chambers A–F contained transfected, induced cells and chambers G and H contained non-transfected parental control cells; all chambers were treated with 10 μM GABA.

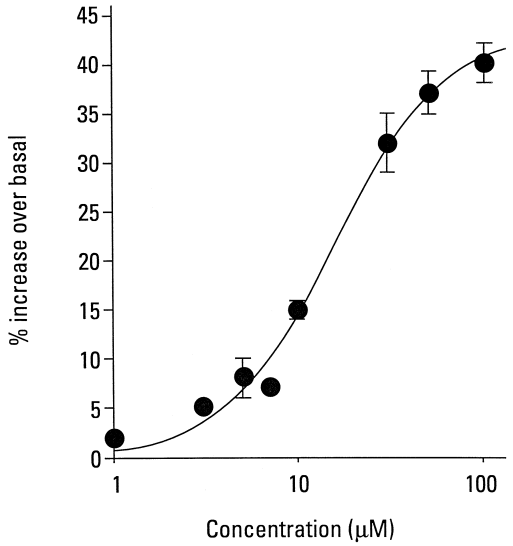


Fig. 2. A representative concentration–response curve to GABA constructed from three independent experiments, showing maximum % increase over baseline. GABA dose-dependently accelerated peak acidification rate in Ltk$^-$ cells expressing human recombinant $\alpha1\beta3\gamma2$ receptors. Results are normalised with respect to baseline and expressed as % increase over control. GABA, 100 μM, produced a 40 ± 2% increase over basal metabolic rate, with EC_{50} of 15.5 μM and Hill slope of 1.5.

found to be optimal for measuring GABA stimulated acidification responses.

3.2. Time-course and optimisation of agonist response

Responses to GABA were characterized by a rapid early alkalinisation (Fig. 1a) followed by a later transient peak of acidification (Fig. 1b), which then returned to baseline. Neither alkalinisation nor acidification responses were observed in non-transfected parental control cells (chambers G and H). The alkalinisation response was time-dependent, appearing early after GABA exposure and therefore being more pronounced with shorter agonist exposure times of 15 s (Fig. 1a). The alkalinisation was also more evident at low agonist doses and became progressively masked at higher concentrations as the metabolic response increased. Time-courses were performed using different agonist exposure times to optimise the acidification response, since this is the response which is produced by all other receptors characterised to date using microphysiometry. Increasing agonist exposure times from 15 to 25 or 30 s prior to rate determination eliminated observation of the early alkalinisation and maximised observation of the later acidification component (Fig. 1b). All subsequent measurements were made under these conditions to minimise observation of the alkalinisation.

A washout period of at least 15 min between successive applications of GABA was found to be necessary to prevent desensitisation-induced decline in responses and a 20 min washout period was routinely used to ensure complete recovery before subsequent tests.

3.3. Effects of GABA on acidification rates

GABA dose-dependently increased extracellular acidifaction rate in cells expressing recombinant $\alpha1\beta3\gamma2$ receptors (Fig. 2) but had no effect in non-transfected parental controls. A maximal concentration of GABA (100 μM) produced an increase of 40 ± 2% ($n = 3$) over basal acidification. A mean dose–response curve constructed from 3 independent experiments revealed an EC_{50} for GABA of 15.5 μM and Hill slope for the mean curve of 1.5.

The effects of GABA on extracellular acidification rates in transfected cells were specifically blocked by the GABA site antagonist bicuculline and the chloride ion channel blocker picrotoxin (100 μM) (picrotoxin data not shown). Fig. 3a shows representative raw data from one of the antagonist experiments with bicuculline, illustrating the basic cyclical pattern of alkalinisation and acidification occurring during pump-on and pump-off periods. Each trace represents a separate chamber with its own population of cells. As can be seen from the traces of non-induced parental cell responses, the pump cycle duration of 1 min allowed time for the pH to recover after acidification and plateau at a steady-state level during washout before the next pump-off period, accumulation of acidic by-products and rate determination. The dual effects of GABA on extracellular pH can be observed in induced cells in Fig. 3a, with a prominent alkalinisation occurring first (pH rises above the plateau) followed by a fall in pH, the rate of which is greater than that observed for the previous pump cycle. In the presence of 30 μM bicuculline, the alkalinisation is no longer evident and the slope of the acidification is less steep, comparable to that seen in the absence of GABA or in non-transfected cells. The GABA concentration-response curve was shifted rightwards in a parallel manner in the presence of the competitive $GABA_A$ receptor antagonist bicuculline (30 μM) (Fig. 3b).

3.4. Allosteric modulation of GABA responses by benzodiazepines and efficacy determination

From the GABA dose–response curve (Fig. 2), a dose of GABA was selected which produced an increase of approximately 20% of the maximum response (10 μM; 11 ± 0.7% increase over basal, $n = 18$) and the modulatory effects of benzodiazepine agonists were examined. Each benzodiazepine site ligand was tested at a fixed concentration relative to its receptor affinity (3 orders of magnitude above K_i). The benzodiazepine full agonist flunitrazepam (1 μM) significantly modulated the response to 10 μM GABA from 11 ± 0.7% to 25 ± 3% increase over basal, a potentiation of 124 ± 15% ($P < 0.001$, $n = 7$) (a single representative experiment is shown in Fig. 4a). In another experiment, a complete concentration response curve to GABA was examined in the presence and absence of 1 μM flunitrazepam. In the presence of the full benzo-

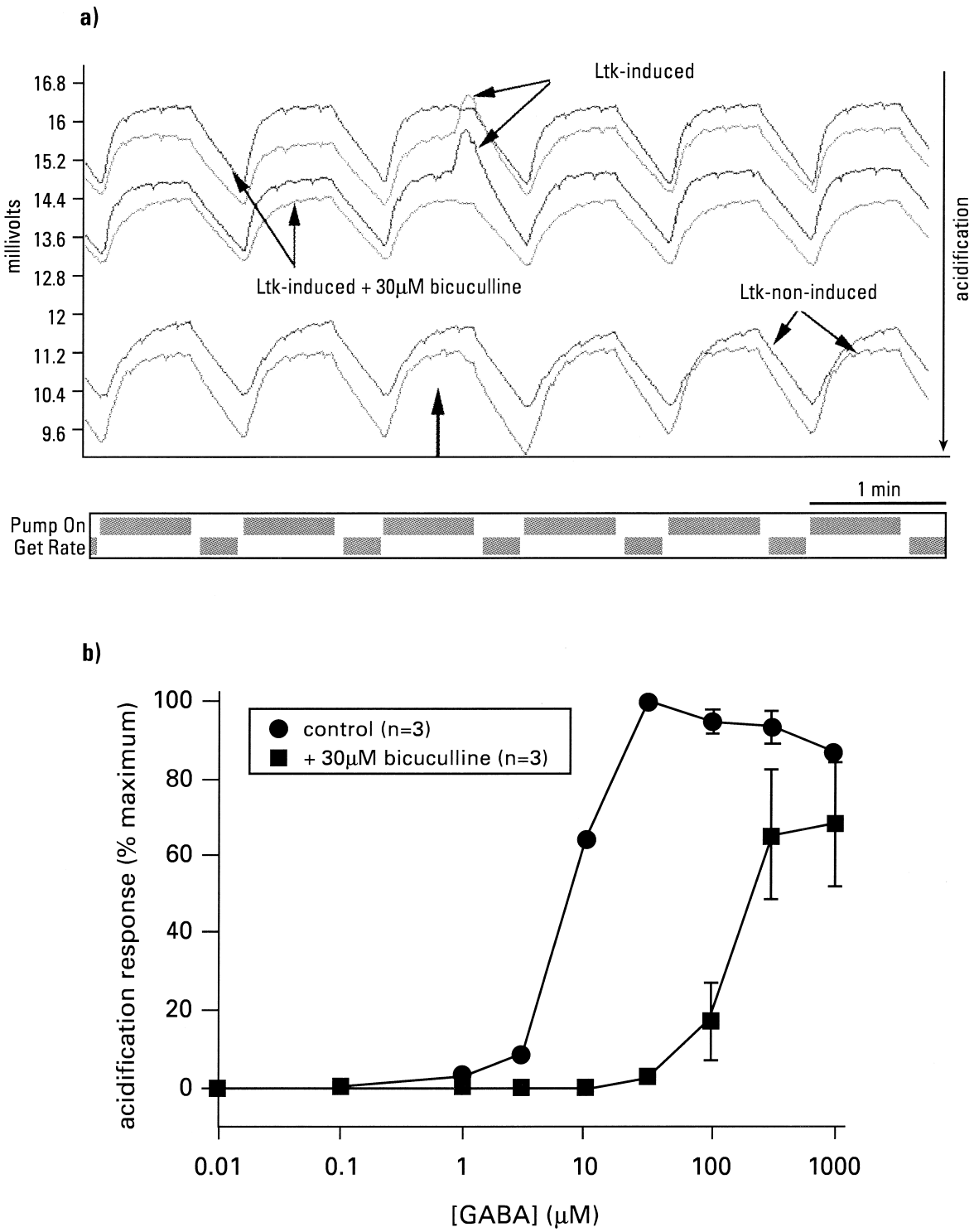
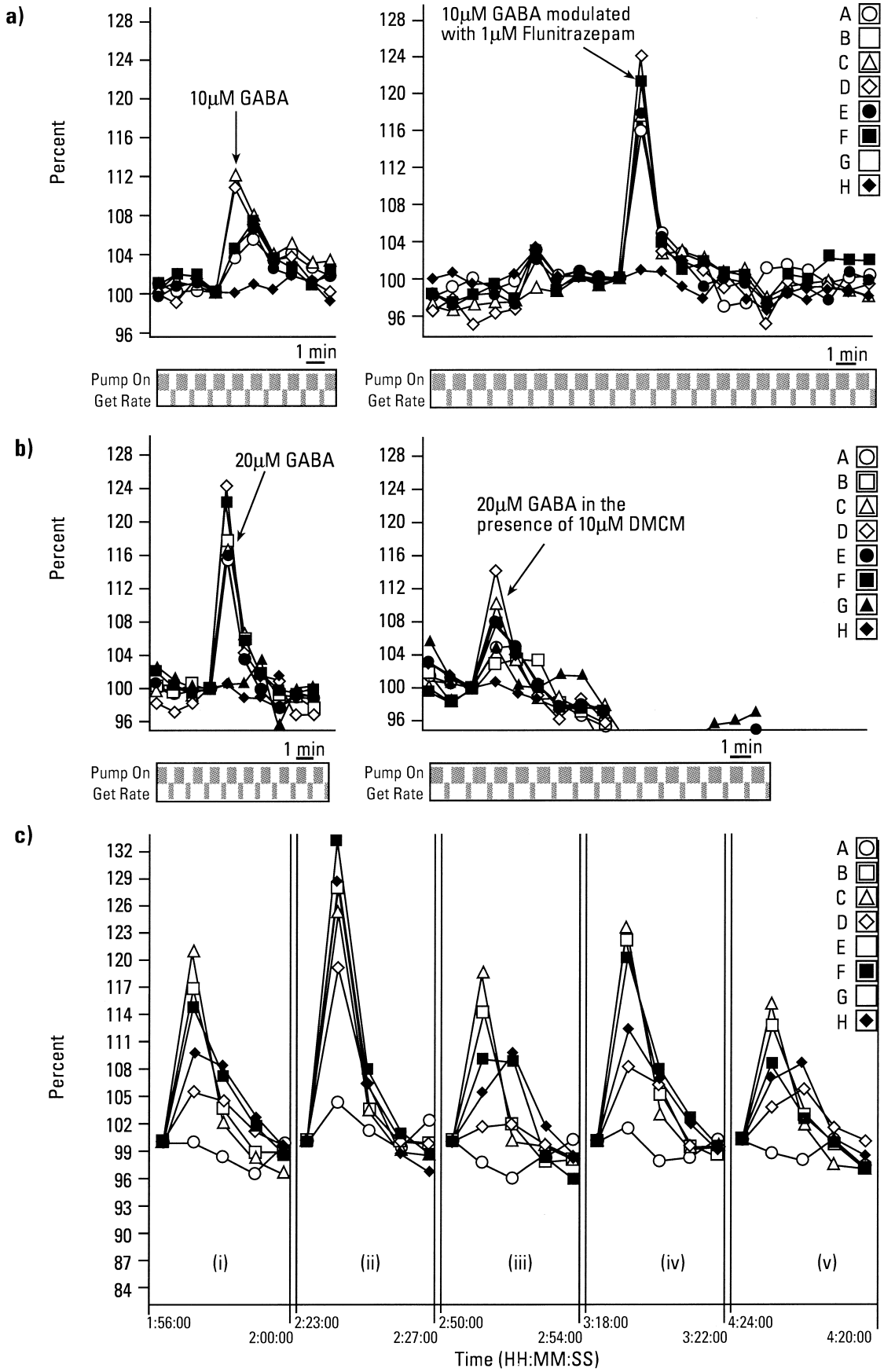


Fig. 3. Antagonism of GABA-induced responses in α1-containing cells. Panel (a) shows typical raw data from a representative experiment with 1 min pump cycle and rate determination between 43 and 58 s. The two lower traces are control responses in non-transfected cells, illustrating the cyclical alkalinisation and acidification which is observed with all cells as the pumps switch on and off. The four upper traces are chambers containing cells expressing functional $GABA_A$ receptors, of which the top and bottom chambers were pretreated with 30 μM bicuculline. 30 μM GABA was applied to all chambers (denoted by arrow) during the pump-on period. The agonist response, observed only in induced cells in the absence of bicuculline, is time-dependent with an early upwards peak of alkalinisation followed by an increased rate of acidification when the pumps switched off. Panel (b) shows a concentration-response curve to GABA ($n = 3$) with competitive antagonism in the presence of 30 μM bicuculline. Data shown in this figure was supplied by Simon Pitchford (Molecular Devices).


a)
10μM GABA
10μM GABA modulated with 1μM Flunitrazepam
Percent
1 min
Pump On
Get Rate
A
B
C
D
E
F
G
H
b)
20μM GABA
20μM GABA in the presence of 10μM DMCM
c)
(i)
(ii)
(iii)
(iv)
(v)
1:56:00
2:00:00
2:23:00
2:27:00
2:50:00
2:54:00
3:18:00
3:22:00
4:24:00
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Time (HH:MM:SS)

Table 1
Comparison between benzodiazepine site ligand efficacy determined using the microphysiometer in stable cell lines expressing recombinant human $\alpha1\beta3\gamma2$ receptors and efficacies determined electrophysiologically at $\alpha1\beta2\gamma2$ receptors expressed in *Xenopus* oocytes

Benzodiazepine site ligand	Cell type		
	$\alpha1\beta3\gamma2$ (Ltk$^-$) K_i (nM)[a]	$\alpha1\beta2\gamma2$ (Oocytes) Electrophysiological efficacy[a] (%)	$\alpha1\beta3\gamma2$ (Ltk$^-$) Microphysiometer efficacy (%)
Flunitrazepam	5.2 ± 0.2	117 ± 7	124 ± 15
Bretazenil	0.35 ± 0.07	29 ± 4	45 ± 13
DMCM	11.2 ± 0.9	-39 ± 4	-53 ± 5
Zolpidem	20.7 ± 2.4	159 ± 30	117 ± 23
Ro15-1788	0.92 ± 0.04	7 ± 9	-1 ± 12

[a] Data taken Hadingham et al., 1996.
Affinities are shown for displacement of 1.8 nM [^{3}H]Ro15-1788 binding and represent the mean ± standard error from at least 3 independent experiments. Electrophysiological data represent maximum modulation of control GABA currents, expressed as mean % potentiation or inhibition ± standard error from at least 4 oocytes. Control GABA responses were obtained by selecting a GABA concentration ~20% of maximum for each individual oocyte, typically 1–10 μM. Microphysiometer data is similarly expressed as % modulation of an ~EC_{20} response to GABA and values are mean ± standard error from at least 3 independent experiments, each with 6 parallel chambers. Binding affinities are also shown.

diazepine agonist the GABA curve was shifted to the left with a 3-fold reduction in the EC_{50} for GABA from 13 to 4 μM (data not shown). Another benzodiazepine site full agonist, zolpidem (10 μM), also produced a large potentiation of the GABA response of $117 \pm 23\%$ ($n = 3$). Benzodiazepine site partial agonists with varying efficacy could also be detected. The partial agonist bretazenil at 100 nM produced a significant potentiation of the response to 10 μM GABA of $45 \pm 13\%$ ($P < 0.01$, $n = 3$) (Fig. 4c). For inverse agonists, which decrease the effectiveness of GABA, a dose of GABA producing ~80% of the maximum response was used to allow modulation in the opposite direction. The benzodiazepine full inverse agonist DMCM (10 μM) inhibited the response to 20 μM GABA by $53 \pm 5\%$ ($n = 3$) (Fig. 4b). In contrast, the benzodiazepine site antagonist Ro15-1788 at 1 μM had no significant efficacy. A comparison of efficacies determined using the microphysiometer and those determined using electrophysiological techniques in oocytes expressing receptors of the same composition is shown in Table 1.

4. Discussion

GABA elicited robust dose dependent increases in extracellular acidification rates in Ltk$^-$ cells expressing $\alpha1\beta3\gamma2$ $GABA_A$ receptors (Fig. 2). The absence of acidification or alkalinisation responses to GABA in non-transfected parental control cells (Fig. 3, panel a) and the specific pharmacological blockade of the GABA responses with the competitive $GABA_A$ receptor antagonist bicuculline (Fig. 3), together with modulations of GABA-induced responses by benzodiazepines, indicate that responses were specifically mediated through the $GABA_A$ receptor ion channel. The dose response curve to GABA was shifted rightwards in a parallel manner in the presence of bicuculline, as would be expected for a competitive antagonist, and although there appeared to be a slight reduction in the maximal response, this was not a significant decrease.

From the mean dose response curve to GABA (Fig. 2), the EC_{50} was determined to be 15.5 μM. In electrophysiological studies in *Xenopus* oocytes expressing recombinant receptors of the same composition ($\alpha1\beta3\gamma2$), the affinity of GABA was 8 ± 2 μM, $n = 5$ (Hadingham et al., 1993), determined using two-electrode voltage-clamp recordings at room temperature. The two assays are therefore in reasonable agreement, despite the fact that measurement of extracellular acidification rate is downstream of direct current measurements. Clearly electrophysiological measurements can offer a more rapid measure of $GABA_A$ receptor ion channel activation whereas use of the microphysiometer necessitates a longer agonist exposure. Desensitization, which is influenced by receptor subunit composition (Tia et al., 1996), is an important consideration for $GABA_A$ receptors (Jones and Westbrook, 1996) with pro-

Fig. 4. A representative experiment illustrating benzodiazepine modulation of the GABA response. Results are rate data normalized with respect to control (a) The benzodiazepine full agonist flunitrazepam (1 μM) significantly potentiated the response to 10 μM GABA (chambers A to G). Chamber H contains non-transfected control cells. (b) The benzodiazepine full inverse agonist DMCM (10 μM) produced a $53 \pm 5\%$ inhibition of the response to 20 μM GABA. Chambers G and H contain non-transfected control cells. (c) A series of normalized segments taken from a representative experiment showing (i) the response to 10 μM GABA (chambers B to H; A contains control cells); (ii) potentiation of this response by the full benzodiazepine agonist zolpidem (1 μM); (iii) recovery of 10 μM GABA response after a 20 min washout period; (iv) potentiation of the GABA response by the benzodiazepine partial agonist bretazenil (100 nM); and finally (v) 10 μM GABA again. Chambers E and G were deleted because of erratic baselines.

longed agonist exposures, and receptors in a desensitized state can result in underestimation of the effective half maximal concentration. However, the closeness of the EC_{50} values determined by electrophysiology and microphysiometry would argue against this being a problem here. The change in extracellular acidification rate represents the net effect of total energy expended by the cell in response to GABA-induced conformational change of the receptor and ion channel opening.

In another recent study using primary cultures of rat cerebellar granule cells, effects of GABA were also maximal at 100 μM, with an EC_{50} of 2 ± 0.2 μM and a maximal effect of $15.4 \pm 1.2\%$ increase over baseline (Brown et al., 1997). The cerebellum contains a mixture of α and γ/δ combinations (% of cerebellar $GABA_A$ receptors): α6γ2 (36%), α6δ (23%), α1γ2 (28%), α2γ1 (8%) and α3γ2 (5%) with no α4, α5 or γ3 (Quirk et al., 1994). Specifically in the granule cell layer, mRNAs have been identified for α1, α6, β2, β3, γ2 and δ subunits (Behringer et al., 1996). Thus the $GABA_A$ receptor population in granule cells is likely to be heterogeneous compared to recombinant α1β3γ2 receptors and this may account for the difference in GABA efficacy between native receptors (EC_{50} 2 μM) and receptors described here (EC_{50} 15.5 μM). A further complication in native tissues may be the presence of $GABA_B$ receptors. The molecular layer of the cerebellum contains high numbers of $GABA_B$ receptors, and whilst the proportion in the granule cell layer is lower ($GABA_A$ 59.6 ± 5.6 pmol/g wet weight, $GABA_B$ 16.8 ± 5.2), they may still be a complicating factor (Bowery et al., 1987). The other notable difference was in the size of the maximum GABA response in the cerebellar granule cells, $15.4 \pm 1.2\%$ vs. $40 \pm 2\%$ here in stable cell lines. This may be a feature of the cerebellar granule GABA response per se (perhaps differences in receptor subtypes) or some other difference between stable cell lines and primary cultures such as differences in basal acidification rates (73 ± 4 μVs^{-1} primary cultures vs. 105 ± 2 μVs^{-1} stable cell lines). Higher receptor densities in stable cell lines are likely to be a key factor.

The time-dependency of GABA responses with two separate components was interesting. The alkalinisation appeared to be $GABA_A$ receptor-specific since it was not observed in non-transfected control cells and was also blocked by the specific $GABA_A$ receptor antagonist bicuculline. The present protocol was optimised for investigation of the acidification response but the molecular basis of the alkalinisation response requires further investigation. Evidence from the literature suggests a possible explanation for this GABA-induced alkalinisation. In addition to chloride ions, $GABA_A$ receptor ion channels also show a significant permeability to bicarbonate ions (Kaila and Voipio, 1987; Kaila et al., 1993). There is an outwardly directed electrochemical driving force for HCO_3^- and GABA channel activation can result in a significant net efflux of HCO_3^- ions through these same channels, leading to extracellular alkalinisation and an influx of CO_2 (Voipio et al., 1995). This HCO_3^-/CO_2 − dependent alkalinisation, which is enhanced by pentobarbital and blocked by picrotoxin (Voipio et al., 1995), can be inhibited by blocking extracellular carbonic anhydrase. Brown et al. (1997) did not report an alkalinisation component in their GABA responses but their longer agonist exposure time would probably have limited observation of this component.

Finally, there was good correlation between benzodiazepine efficacies determined electrophysiologically (Table 1) and those measured using microphysiometry for a variety of standard benzodiazepine ligands with a range of efficacies. Agonist and inverse agonist allosteric modulation was demonstrated and results were quantitatively what would be predicted from the intrinsic efficacy of the benzodiazepine site ligands (antagonist, partial agonist and full agonists) at this receptor subunit combination.

In summary, the microphysiometer offers an alternative functional assay for $GABA_A$ receptors. It can be used to measure gross changes in ion channel activity and also to reliably measure the subtle modulatory effects on ion channel conductance of allosteric modulators and further, to determine their relative efficacy. Additionally, the current methodology in stable cell lines allows the flexibility to examine various specific combinations of receptor subunits.

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