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Few Cell Lines with $GABA_A$ mRNAs Have Functional Receptors

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In the preceding companion article (Tyndale et al., 1994) we used the PCR to investigate the occurrence of 13 $GABA_A$ receptor subunit mRNAs in several cell lines, including those derived from brain (B65, B104, and NB41A3), cerebellum (C17), glia (C6), pituitary (AtT-20), adrenal medulla (PC12), and the endocrine pancreas (RINm5F and βTC3). In the present study we used the whole-cell configuration of the patch-clamp technique to determine which of these cell lines express functional $GABA_A$ receptors. All of the cell lines contain detectable levels of at least one $GABA_A$ receptor subunit mRNA (Tyndale et al., 1994); however, only RINm5F and βTC3 cells exhibited GABA-evoked currents. GABA activated currents in all RINm5F cells, but currents were only barely detectable in 50% of βTC3 cells tested. Many of the cell lines that failed to respond to GABA were derived from cell types with functional $GABA_A$ receptors. For example, the failure of PC12 cells to respond to GABA contrasts with the observation of GABA responses recorded from all primary cultured adrenomedullary chromaffin cells tested. GABA-evoked currents recorded from βTC3 cells were too small (< 10 pA) to characterize pharmacologically. However, GABA activated robust currents recorded from RINm5F cells. These currents reversed at a holding potential similar to the equilibrium potential for Cl^-, were blocked by the antagonist bicuculline methiodide (10 μM), and were potentiated by pentobarbital (100 μM). RINm5F cell $GABA_A$ receptors were insensitive to diazepam (10 μM) and were inhibited by Zn^{2+} (10 μM). For comparison, diazepam (5 μM) caused a large potentiation and Zn^{2+} (100 μM) only a relatively small inhibition of GABA responses recorded from primary cultured chromaffin cells. Benzodiazepine modulation is dependent on the γ $GABA_A$ receptor subunit and Zn^{2+} inhibits only those receptors lacking the subunit. Hence, taken together these observations suggest that in contrast to chromaffin cells, RINm5F cells express functional $GABA_A$ receptors that lack γ subunits. Many neuronal and peripheral cell lines express $GABA_A$ receptor mRNAs, but few have functional receptors. The occurrence of functional $GABA_A$ receptors in cell lines is discussed in relation to their tissues of origin.

[Key words: $GABA_A$ receptors, endocrine pancreas, patch clamp, chromaffin cells, RINm5F cells, βTC3 cells, PC12 cells, AtT-20 cells, C6 cells, C17 cells, B65 cells, B104 cells, NB41A3 cells]

GABA type A ($GABA_A$) receptors are widely distributed throughout the mammalian nervous system, where their role is predominantly inhibitory. GABA binds to its heterooligomeric receptor causing a conformational change, which allows Cl^- ions to flow across cell membranes, down their electrochemical gradient. This process is modulated by a plethora of therapeutically important compounds including the benzodiazepines and most general anesthetics (Hales and Olsen, 1994).

By analogy with the nicotinic ACh receptor (Unwin, 1989), the $GABA_A$ receptor is thought to be composed of five subunits (Olsen and Tobin, 1990). With at least 15 different mammalian subunits (α1-6, β1-3, γ1-3, δ, ρ1, and ρ2) there is clearly potential for numerous $GABA_A$ receptor subtypes (Burt and Kamatchi, 1991). Different receptor subunit combinations have disparate pharmacological (Draguhn et al., 1990; Pritchett and Seeburg, 1990; Wafford et al., 1991) and biophysical properties (Angelotti and Macdonald, 1993). Moreover, immunoblotting, protein chemistry, and binding studies provide evidence for the expression of different $GABA_A$ receptor subtypes in the nervous system (Bureau and Olsen, 1990; Olsen et al., 1990; Whiting et al., 1990).

In addition to their location on central neurons and astroglia (Bormann and Kettenmann, 1988), functional $GABA_A$ receptors are also found in peripheral neurons (Bowery and Brown, 1974) and non-neuronal cells. Peripheral cells with $GABA_A$ receptors include melanocyte-stimulating hormone secreting cells of the pituitary pars intermedia (Demeneix et al., 1986), catecholamine-secreting chromaffin cells of the adrenal medulla (Bormann and Clapham, 1985; Peters et al., 1989), and glucagon secreting α-cells of the islets of Langerhans (Rorsman et al., 1989). Although functional $GABA_A$ receptors are present in numerous primary cultured cells, there have only been two reports of cell lines expressing GABA-gated Cl^- ion channels (Hales et al., 1992; Anderson et al., 1993).

Clonal cell lines are valuable tools for molecular biological, biochemical and electrophysiological studies of many neurotransmitter receptors. For example, neuroblastoma–glioma lines have been extensively used for characterizing and cloning the ligand-gated 5-HT_3 (Lambert et al., 1989; Maricq et al., 1991) and the G-protein coupled δ-opioid (Evans et al., 1992) receptors. Once receptors are cloned, cell lines are also useful for studying the regulation of receptor transcription and expression.

The dearth of cell lines available for the study of $GABA_A$ receptors prompted our extensive investigation of 13 $GABA_A$

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receptor subunits using the PCR in many cell lines, including cerebellar (C17), brain (B65, B104), neuroblastoma (NB41A3), glioma (C6), pituitary (AtT-20), pheochromocytoma (PC12) cells, and RINm5F and βTC3 cell lines derived from the endocrine pancreas (Tyndale et al., 1994). The preceding companion article (Tyndale et al., 1994) demonstrates that these cells contain many different $GABA_A$ receptor subunit mRNAs. In the present article we used the whole-cell configuration of the patch-clamp technique to determine whether these cells express functional $GABA_A$ receptors. Of nine cell lines known to contain $GABA_A$ receptor mRNAs, GABA activated currents in only two, the βTC3 and RINm5F cell lines.

Materials and Methods

Cell line cultures. C17, B65, B104, NB41A3, C6, AtT-20, PC12, RINm5F, and βTC3 cell lines were cultured in the appropriate growth medium in 75 cm^2 tissue culture flasks (Costar) as described in the preceding companion article (Tyndale et al., 1994). Having reached approximately 80% confluency, cells were removed by replacing the culture medium with 2.5 ml of Ca^{2+}-, Mg^{2+}- and bicarbonate-free Hank's solution, containing 500 mg/liter trypsin, 200 mg/liter EDTA, and 10 mg/liter phenol red (Irvine Scientific). To prevent excessive digestion, after 10 min, 10 ml of culture medium was added to the cell suspension and cells were washed by centrifugation and resuspension in 10 ml of fresh medium. For electrophysiological studies, 0.1 ml of the cell suspension was added to 2 ml of culture medium in 35 mm diameter dishes (Costar) and grown for 3–7 d prior to experimentation.

Chromaffin cell cultures. Fresh bovine adrenomedullary glands were provided by the local abattoir (Shamrock Meats Inc., Vernon, CA), flushed with ice-cold Ca^{2+} free phosphate-buffered saline (PBS; GIBCO) containing heparin (1 U/liter) and transported to the laboratory on ice. The medulla was dissected away from the surrounding cortex, cut into fragments, and placed into a dissociation medium composed of PBS with Ca^{2+} (1 mM) and collagenase type 1 (1 mg/ml; Sigma). The suspension was incubated (45 min, 37°C) in a shaking water bath. Every 15 min the partially dissociated tissue was triturated with a wide (2 mm) bore pipette. After incubation the suspension was filtered through a 70 μm fluorocarbon filter (Spectrum, Houston, TX) and the filtrate was washed twice by centrifugation and resuspension in Ca^{2+}-free PBS containing 5 mg/ml bovine serum albumin (BSA; Sigma). The pellet was finally resuspended in 1 ml of the washing solution and pipetted onto the top of a "cushion" of BSA (25 mg/ml) in Ca^{2+} free PBS. After 90 min the cushion was carefully aspirated leaving a pellet composed of predominantly viable chromaffin cells. The pellet was resuspended in a Dulbecco's modified Eagle's medium (GIBCO) based growth medium supplemented with 10% (v/v) calf serum, penicillin (50 IU/liter) and streptomycin (50 μg/ml). Cells liberated from one gland were plated into eight 35 mm diameter dishes. Chromaffin cells were used for electrophysiological experiments after 1–4 d of incubation in an humid atmosphere of 95% air, 5% CO_2 at 37°C.

Electrophysiology. Using the whole-cell configuration, currents were recorded with a List Electronics L/M EPC7 patch-clamp amplifier (Hamill et al., 1981). Cells were continuously superfused (1.5 ml/min) with extracellular solution consisting of (in mM) NaCl, 140; KCl, 2.8; $MgCl_2$, 2.0; $CaCl_2$, 1.0; glucose, 6; and HEPES-NaOH, 10 (pH 7.2). The electrode solution contained (in mM) CsCl, 140; $MgCl_2$, 2.0; $CaCl_2$, 0.1; EGTA, 1.1; ATP (Mg^{2+} salt), 3; and HEPES-CsOH, 10 (pH 7.2). Junction potentials were nulled with an open electrode in the recording chamber prior to each experiment. GABA (100 μM) was applied by pressure ejection (1.4×10^5 Pa for 10–1000 msec at 0.04 Hz) from modified patch pipettes. Other compounds were bath applied. Experiments were carried out at 20–24°C. Currents were filtered (1 kHz low-pass, 8-pole Bessel; Frequency Devices, D02LPF), recorded on chart paper (Gould, Brush 2200), and simultaneously acquired (Labmaster DMA) and digitized (4 kHz) for storage on the hard disk of an IBM PC. Currents were averaged, superimposed, and measured using pCLAMP software (Axon Instruments, Burlingame, CA). Cells, voltage clamped at −60 mV, were considered to respond to GABA if the current record deviated from baseline by >5 pA upon GABA application.

Drugs used. γ-Aminobutyric acid (GABA), acetylcholine chloride (ACh), bicuculline methiodide, sodium pentobarbital, and diazepam were all from Sigma. Stock solutions of diazepam, in ethanol, were diluted to achieve an ethanol concentration of <0.1%.

Results

Sensitivity of cell lines to GABA

Cerebellar (C17), brain (B65, B104), neuroblastoma (NB41A3), glioma (C6), pituitary (AtT-20), pheochromocytoma (PC12), and pancreatic (RINm5F, βTC3) cell lines were tested for functional $GABA_A$ receptors (Table 1). Cells were voltage clamped at −60 mV using the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981). GABA (100 μM) was applied by pressure (1.4×10^5 Pa, 10 msec duration) ejection from a glass micropipette positioned within 50 μm of the cell. When cells failed to respond to GABA, the duration of application was increased (up to 1 sec). To prevent desensitization of $GABA_A$ receptors, applications were separated by at least 20 sec and cells were continuously superfused with fresh extracellular solution. None of the C17 (n = 5), B65 (n = 8), B104 (n = 10), NB41A3 (n = 10), C6 (n = 15), AtT-20 (n = 10) and PC12 (n = 15) cells tested responded to GABA application (Table 1), despite these cell lines all containing detectable levels of $GABA_A$ receptor subunit mRNAs (Tyndale et al., 1994).

PC12 cells have been reported to express nicotinic ACh receptors (Bormann and Matthaei, 1983). Many of the PC12 cells that did not respond to GABA were subsequently tested with ACh. The GABA (100 μM)-containing micropipette was removed from the bath and replaced with one containing ACh (100 μM). Although none of the PC12 cells responded to GABA, subsequent pressure application of ACh (1.4×10^5 Pa, 10 msec duration) rapidly activated inward currents in all cells tested (n = 5, data not shown). In contrast to PC12 cells, 100% of primary cultured bovine chromaffin cells (n = 20), derived from the adrenal medulla, responded electrophysiologically to GABA (see Fig. 3*C,D*). However, in common with the PC12 cells, all chromaffin cells tested also responded to ACh (100 μM; n = 10). These data confirm earlier reports (Peters et al., 1989) of functional $GABA_A$ and ACh receptors in individual chromaffin cells.

Of the cell lines tested, only RINm5F and βTC3 cell lines, derived from the endocrine pancreas, had discernable responses to GABA (Table 1). GABA-evoked currents were only observed in 50% of βTC3 cells tested (n = 12); the peak currents (<10 pA) were too small to characterize pharmacologically. However, in two cells GABA-evoked currents were observed to reverse in direction at a membrane potential close to 0 mV (data not shown). With equal Cl^- either side of the cell membrane, the reversal potential of GABA-activated currents recorded from βTC3 cells was close to the Cl^--equilibrium potential (E_{Cl^-}). This observation supports the hypothesis that βTC3 cells express low levels of functional $GABA_A$ receptors.

Application of GABA (100 μM) to RINm5F cells rapidly activated clearly discernable inward currents at a holding potential of −60 mV (n = 40; Fig. 1*A*). Increasing the duration of GABA application increased the amplitude of GABA-evoked currents recorded from RINm5F cells (n = 40). Peak GABA-evoked currents ranged from 15 to 100 pA, with a mean amplitude of 32 ± 7 pA (mean ± SEM, n = 13).

Characterization of GABA-evoked currents recorded from RINm5F cells

GABA-evoked currents recorded from RINm5F cells became smaller when the membrane potential was shifted from −60 mV toward 0 mV (Fig. 1). Currents reversed from inward to

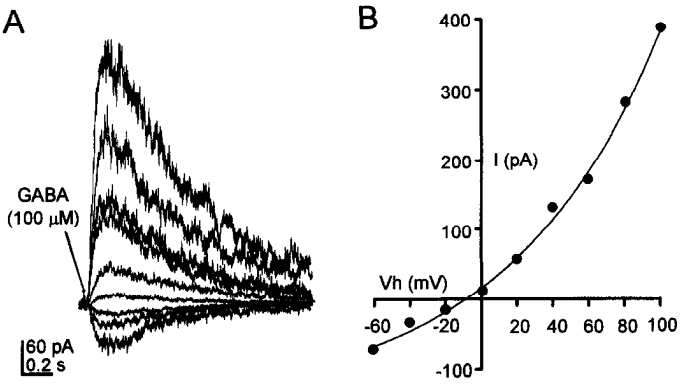

Figure 1. GABA-activated currents recorded from RINm5F cells reverse at the Cl^- equilibrium potential. *A*, Currents activated by GABA (100 μM) recorded, at holding potentials between −60 and 100 mV, from a RINm5F cell with equal Cl^- either side of the cell membrane. Superimposed currents represent averages of two responses recorded at each potential in response to pressure applied GABA (1.4×10^5 Pa, for 15 msec at a frequency of 0.04 Hz). All currents were recorded from the same cell and were low-pass filtered at 1 kHz. *B*, Graph of the relationship between current amplitude (pA) and holding potential (mV) for the responses illustrated in *A*. RINm5F cell GABA responses exhibit outward rectification similar to that observed during whole-cell recordings from other preparations (see text). The curve was fitted to data points with an exponential function. Reversal potentials were directly observed as the membrane potential at which no current was elicited by GABA. The mean reversal potential calculated from four such experiments was 1.3 ± 4.9 mV, close to the equilibrium potential for Cl^- calculated using the Nernst equation (0 mV).

outward at a potential of 1.3 ± 4.9 mV (n = 4) and became relatively large at more depolarized potentials (Fig. 1). Hence, like GABA-evoked currents recorded from βTC3 cells, GABA-elicited currents in RINm5F cells have a reversal potential similar to E_{Cl-}. The outward rectification of GABA-evoked whole-cell currents evident in Figure 1 was a feature of all RINm5F cells tested (n = 4) and resembles that reported for various other cell types including primary cultured chromaffin cells (Peters et al., 1989) and the GT1-7 cell line (Hales et al., 1992).

GABA (100 μM)-activated Cl^- currents recorded from RINm5F cells were reversibly blocked (n = 4) by bath application of the selective $GABA_A$ receptor antagonist bicuculline methiodide (10 μM; Fig. 2*A*). $GABA_A$ receptors are positively modulated by a variety of compounds with central depressant activity. These compounds include the anesthetic barbiturates (Barker and Ransom, 1978; Simmonds, 1981) and the benzodiazepines (Macdonald and Barker, 1978). Bath application of the anesthetic barbiturate pentobarbital (100 μM) caused an increase in the amplitude of GABA-evoked currents recorded from RINm5F cells to 317 ± 89% (n = 6) of control (Fig. 2*B*). Both the inhibition of GABA-activated Cl^- currents by bicuculline methiodide and their potentiation by pentobarbital confirm that RINm5F cells express functional $GABA_A$ receptors.

Comparison of diazepam and Zn^{2+} actions on RINm5F and chromaffin cell $GABA_A$ receptors

In contrast to the marked enhancement elicited by pentobarbital, bath application of the benzodiazepine diazepam (10 μM) had no effect (n = 5) on the amplitude of GABA-activated currents (Figs. 3*A*, 4) recorded from RINm5F cells. Primary cultured bovine adrenomedullary chromaffin cells, which are known to express functional $GABA_A$ receptors (Bormann and Clapham, 1985; Peters et al., 1989) were examined for comparison with RINm5F cells. Figure 3*C* illustrates the increase in amplitude of GABA-evoked currents recorded from a chromaffin cell when a lower concentration of diazepam (5 μM) was bath applied. Diazepam caused a potentiation of GABA-activated currents recorded from chromaffin cells to 214 ± 25% (n = 5) of control amplitude (Fig. 4).

Table 1. Summary of the sensitivity of cell lines to GABA

Origin	Cell lines	GABA-evoked currents?
Periphery		
Endocrine pancreas	RINm5F	40/40
	βTC3	6/12
Adrenal medulla	PC12	0/15
Pituitary	AtT20	0/10
Brain		
Neuroblastoma	B65	0/8
	B104	0/10
	NB41A3	0/10
Cerebellum	C17	0/5
Glia	C6	0/15

GABA application to nine cell lines, derived from the periphery and brain, activated currents only in cells originating from the endocrine pancreas. Single cells were voltage clamped in the whole-cell configuration of the patch-clamp technique and GABA (100 μM) was pressure applied (see Materials and Methods). The table summarizes the regions from which the cell lines were derived and the number of cells that responded alongside the number of each cell type tested. GABA-evoked currents were detectable in only 50% of βTC3 cells tested whereas 100% of RINm5F cells exhibited GABA-activated currents. Interestingly, all these cell lines have detectable levels of $GABA_A$ receptor subunit mRNAs (Tyndale et al., 1994).

Benzodiazepines only modulate $GABA_A$ receptors incorporating γ subunits (Pritchett et al., 1989; Ymer et al., 1990; Knoflach et al., 1991). In addition, Zn^{2+} inhibits GABA responses recorded from cells lacking γ $GABA_A$ subunits (Draguhn et al., 1990; Smart et al., 1991). Bath application of Zn^{2+} (10 μM and 100 μM) caused a dose-dependent inhibition of GABA-evoked currents (Fig. 3*B*). Zn^{2+} (10 μM) inhibited GABA-activated currents to 45 ± 10% (n = 4) of control amplitude (Fig. 4). The sensitivity of chromaffin cell $GABA_A$ receptors to Zn^{2+} was examined for comparison with RINm5F cell receptors. In contrast to the inhibition by Zn^{2+} observed in RINm5F cells, Zn^{2+} (10 and 100 μM) caused only a relatively small attenuation of GABA-activated currents recorded from chromaffin cells (Fig. 3*D*). Zn^{2+} (100 μM) reduced the GABA-evoked current amplitude, recorded from chromaffin cells, to 67 ± 8% (n = 4) of control amplitude (Fig. 4). The Zn^{2+} (100 μM)-induced inhibition of $GABA_A$ receptors in chromaffin cells is smaller than that observed when a 10-fold lower dose of the ion was applied to RINm5F cells (Fig. 4). These data are consistent with chromaffin cells and RINm5F cells expressing $GABA_A$ receptors with and without γ subunits, respectively.

Discussion

Our previous study tested 13 cell lines for 13 $GABA_A$ receptor subunit mRNAs using PCR (Tyndale et al., 1994). In this study we tested B65, B104, NB41A3, C17, C6, AtT-20, PC12, βTC3, and RINm5F cell lines for functional $GABA_A$ receptors using the whole-cell configuration of the patch-clamp technique. Despite all the cell lines having detectable levels of $GABA_A$ receptor

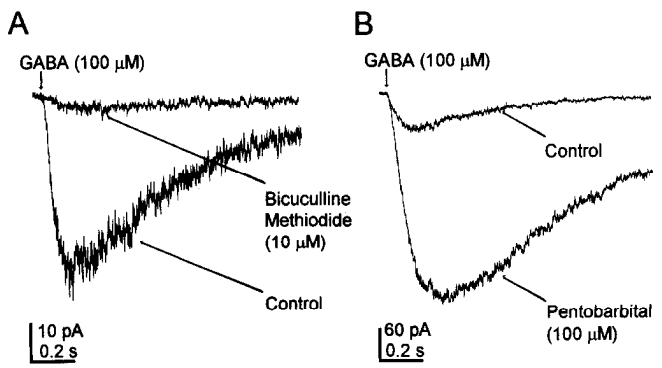

Figure 2. GABA-evoked currents recorded from RINm5F cells exhibit $GABA_A$ receptor pharmacology. *A*, GABA (100 μM)-activated currents recorded from RINm5F cells before and during bath application of bicuculline methiodide (10 μM). Currents were reversibly abolished by the $GABA_A$ receptor–specific antagonist in the exemplar cell and three additional cells tested. *B*, Currents evoked by GABA (100 μM) were potentiated by bath application of the anesthetic barbiturate pentobarbital (100 μM). Pentobarbital caused a marked enhancement of the amplitude and duration of GABA-activated currents (n = 6). Superimposed traces are averages of five currents recorded under control conditions and in the presence of either drug. Data in *A* and *B* were recorded from separate RINm5F cells and were low-pass filtered at 1 kHz.

mRNAs (Tyndale et al., 1994), only the pancreatic cells βTC3 and RINm5F responded to GABA. GABA-activated currents recorded from βTC3 cells were too small to characterize pharmacologically. However, consistent with GABA activating $GABA_A$ receptor-Cl^--channels in βTC3 cells, GABA-evoked currents had reversal potentials similar to the Cl^--equilibrium potential. GABA activated more robust Cl^- currents when applied to RINm5F cells, allowing their pharmacological characterization. The inhibition of GABA-evoked currents by bicuculline methiodide confirms that RINm5F cells express $GABA_A$ receptors. This is only the third cell line identified with functional $GABA_A$ receptors (Hales et al., 1992; Anderson et al., 1993).

Pharmacological properties of RINm5F cell $GABA_A$ receptors

The depressant barbiturates and benzodiazepines increase binding of ^{3}H-muscimol to $GABA_A$ receptors and these, and various other compounds with general anesthetic properties, greatly enhance GABA-activated Cl^- currents recorded from neurons and chromaffin cells (see Hales and Olsen, 1994). The anesthetic barbiturate pentobarbital also markedly potentiates GABA-evoked currents recorded from RINm5F cells. However, $GABA_A$ receptors of the pancreatic cell line were insensitive to the anxiolytic benzodiazepine diazepam. $GABA_A$ receptors lacking γ subunits are insensitive to benzodiazepines (Pritchett et al., 1989; Ymer et al., 1990; Knoflach et al., 1991). The $GABA_A$ receptor modulatory action of Zn^{2+} also discriminates between receptors with and without γ subunits. Zn^{2+} inhibits current through $GABA_A$ receptor Cl^- channels that do not contain the γ subunit, while currents through channels containing the γ subunit are insensitive to the Zn^{2+} (Draguhn et al., 1990; Smart et al., 1991). Consistent with RINm5F cells expressing $GABA_A$ receptors without γ subunits, GABA-evoked currents recorded from the pancreatic cell line were attenuated by low concentrations of Zn^{2+}.

Using PCR to identify $GABA_A$ receptor mRNAs, in contrast to rat brain controls, no γ subunit mRNAs were detected in RINm5F cells after 30 amplification cycles (Tyndale et al., 1994). These data correlate well with the lack of γ subunit pharmacology displayed by functional $GABA_A$ receptors of RINm5F cells. However, after 40 amplification cycles γ1-3 mRNAs were apparent in the cell line (Tyndale et al., 1994). It is therefore possible that γ subunits are present in RINm5F cells either in nonfunctional receptor combinations, or perhaps contributing to only a small portion of the functional receptors.

Many cell lines with $GABA_A$ receptor mRNAs, few with functional receptors

A wide variety of cell lines with diverse origins were examined in this study. In the preceding companion article we determined that all of these cell lines contain detectable levels of $GABA_A$ receptor mRNAs (Tyndale et al., 1994). However, of the nine cell lines tested in the present study, and five cell lines tested in previous studies (Hales et al., 1992; Kasckow et al., 1992; Anderson et al., 1993), only three express functionally characterized $GABA_A$ receptors. These three cell lines are immortalized GnRH-secreting hypothalamic (GT1-7) neurons (Hales et al., 1992), human neuroblastoma (IMR-32) cells (Anderson et al., 1993), and now, RINm5F tumoral pancreatic cells.

It is surprising that many of the cell lines, thought to originate from cell types known to express $GABA_A$ receptors, fail to respond to GABA. For example, GABA activates Cl^- currents recorded from primary cultured glia (Bormann and Kettenmann, 1988) and chromaffin cells (Bormann and Clapham, 1985; Peters et al., 1989; present results). However, C6 glioma cells, and PC12 cells derived from the adrenal medulla, do not respond to GABA. In addition, although $GABA_A$ receptors are the major inhibitory neurotransmitter receptors in the brain, the centrally derived B65, B104, NB41A3, and C17 cell lines tested here, failed to respond electrophysiologically to GABA.

The cell lines lacking functional $GABA_A$ receptors are not simply devoid of all ion channels; for example, PC12 cells respond to ACh and, upon depolarization, NB41A3 cells exhibited TTX-sensitive Na^+ current (Hales and Tyndale, unpublished observations). Therefore, at least some of the cell lines lacking functional $GABA_A$ receptors have the ion channels characteristic of their cells of origin. In addition, immunoblotting demonstrates that some of the cell lines without functional receptors, but with $GABA_A$ receptor mRNAs, have receptor subunit protein associated with their membranes (Kasckow et al., 1992); possible reasons why these cells fail to respond to GABA are discussed in the preceding companion article (Tyndale et al., 1994).

Properties of functional $GABA_A$ receptors on cell lines

There are similarities between the pharmacological properties of $GABA_A$ receptors expressed by GT1-7, IMR-32, and RINm5F cell lines. Patch-clamp recordings of GABA-evoked Cl^- currents reveal that, like RINm5F cells, GT1-7 cells express $GABA_A$ receptors positively modulated by pentobarbital, but insensitive to diazepam and inhibited by Zn^{2+}. Likewise, GABA-activated Cl^- efflux from IMR-32 cells is enhanced by the barbiturate but insensitive to benzodiazepines (Anderson et al., 1993). Additionally, ^{3}H-flunitrazepam does not bind specifically to GT1-7 or IMR-32 membranes, demonstrating a lack of central benzodiazepine receptors (Hales et al., 1992; Anderson et al., 1993). These data are consistent with all three cell types expressing functional $GABA_A$ receptors deficient in γ subunits.

GABA activates relatively modest peak currents when applied

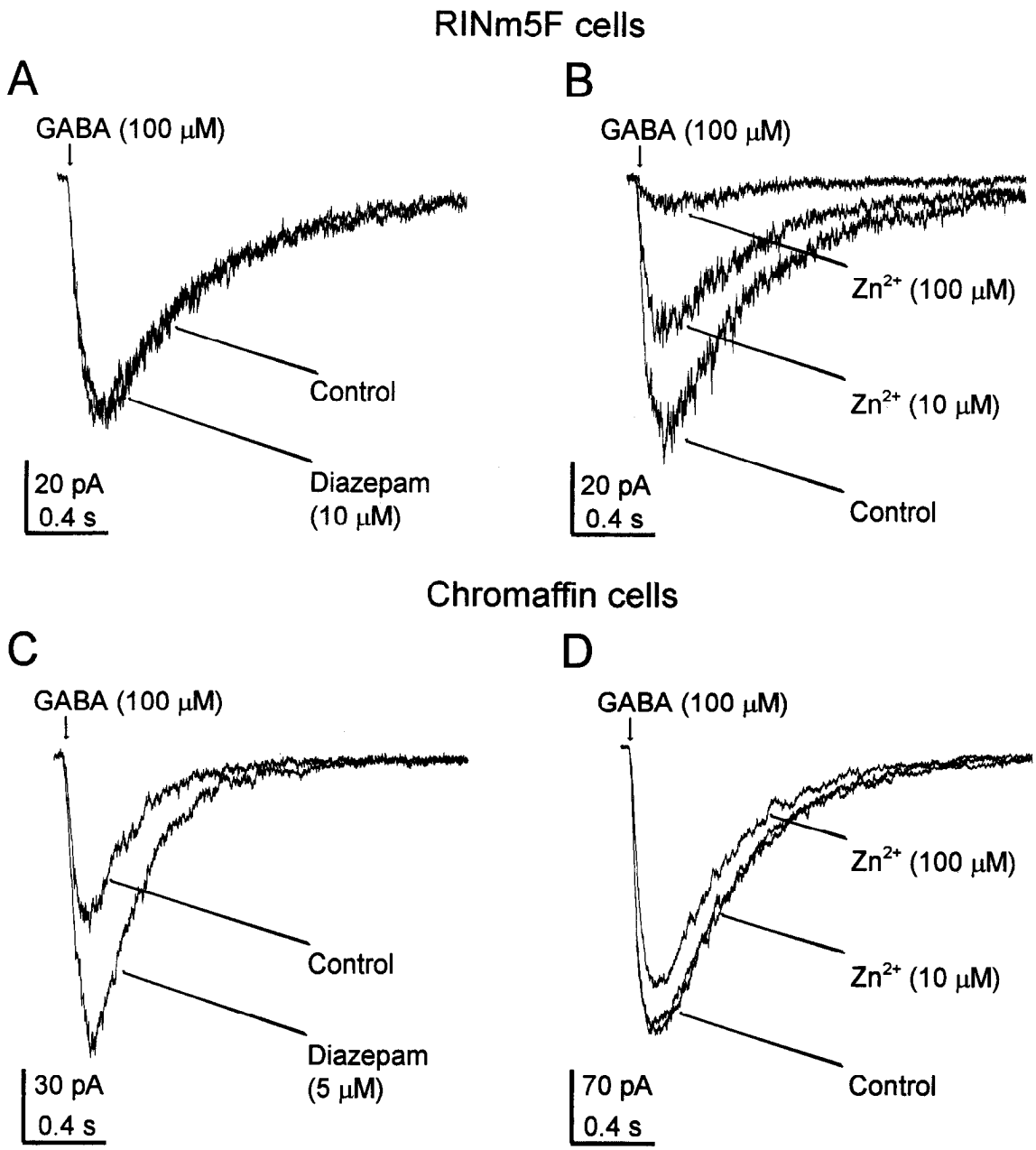

Figure 3. Modulation of $GABA_A$ receptors in RINm5F and chromaffin cells by diazepam and Zn^{2+}. *A*, GABA (100 μM)-activated currents recorded from RINm5F cells before and during bath application of diazepam (10 μM) have similar amplitudes. $GABA_A$ receptors in this cell and three other RINm5F cells were insensitive to the benzodiazepine. *B*, Bath applied Zn^{2+} (10 and 100 μM) inhibited GABA (100 μM)-evoked currents recorded from RINm5F cells. The inhibition by Zn^{2+} was reversible and dose dependent. Superimposed traces in *A* and *B* are the averages of four currents recorded from the same cell and low-pass filtered at 1 kHz. *C*, Currents activated by GABA (100 μM) recorded from a bovine adrenomedullary chromaffin cell before and during bath application of diazepam (5 μM). The benzodiazepine increased the GABA-evoked current amplitude. *D*, Zn^{2+} (10 μM), applied to the bath, had no effect on the amplitude of GABA (100 μM)-activated currents recorded from chromaffin cells. A higher dose of Zn^{2+} (100 μM) caused a small inhibition of the GABA-evoked current. Superimposed traces in *C* and *D* are averages of four currents recorded from the same chromaffin cell and low-pass filtered at 1 kHz.

to GT1-7 (Hales et al., 1992, 1994) and RINm5F cells, and GABA-activated currents are barely detectable in βTC3 cells. In all cases GABA-evoked current amplitudes were smaller than those observed in untransformed neuronal and chromaffin cell preparations (Barker et al., 1982; Peters et al., 1989). There are either fewer $GABA_A$ receptors in the cell lines than in the primary cell preparations, or the GABA-gated Cl^- channels in the former have a smaller conductance. The mean conductance for GABA-gated ion channels in GT1-7 cells (Hales et al., 1994), revealed by variance analysis, is similar to that reported for nontransformed neuronal preparations (Barker et al., 1982; Gold and Martin, 1984). Therefore, the small peak amplitude of GABA-activated currents recorded from GT1-7 cells is consistent with fewer functional receptors in these cells compared to nontransformed preparations. Likewise, although the conductance of GABA-gated channels in RINm5F cells has not been calculated, the small peak amplitude of GABA-evoked currents recorded from these cells suggests that they have rel-

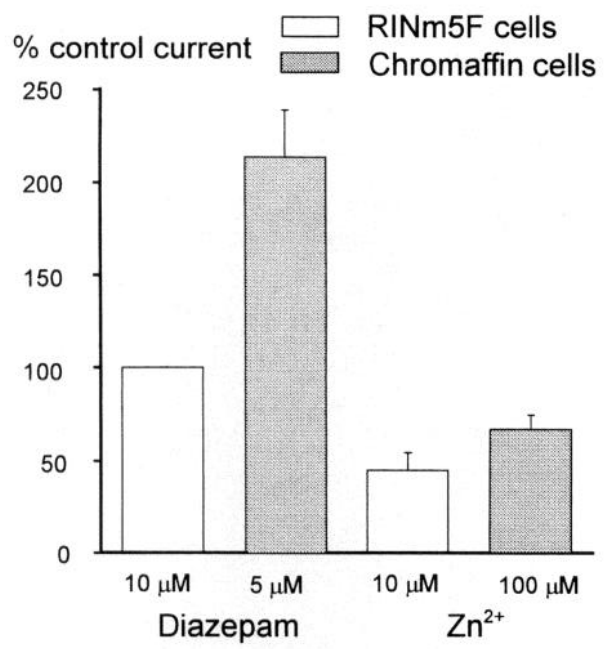

Figure 4. $GABA_A$ receptors of chromaffin cells have γ subunit–specific pharmacology; those of RINm5F cells do not. The graph illustrates mean data obtained from at least four cells. The concentrations of diazepam and Zn^{2+} tested on RINm5F and chromaffin cells are displayed on the *abscissa*; the *ordinate* indicates the GABA (100 μM)-evoked current amplitude in the presence of each agent as a percentage of the control current amplitude (error bars represent +SEM). GABA responses recorded from RINm5F cells were unaffected by 10 μM diazepam, while 5 μM diazepam caused a marked increase in the amplitude of currents recorded from chromaffin cells. In contrast, bath application of 10 μM Zn^{2+} to RINm5F cells more substantially depressed GABA-activated currents than did bath application of 100 μM Zn^{2+} to chromaffin cells. Taken together, these data suggest that chromaffin cells express functional $GABA_A$ receptors with γ subunits, while those of RINm5F cells lack the subunit (see Results).

atively few functional receptors. Pancreatic βTC3 cells had barely detectable GABA-evoked currents, too small to be fully characterized. These cells have $GABA_A$ subunit mRNAs (Tyndale et al., 1994), and our data suggest that they also express low levels of functional receptors. The amount of $GABA_A$ receptor subunit transcript, revealed by the PCR product, is smaller in the pancreatic cell lines relative to rat brain, consistent with the cell lines expressing fewer $GABA_A$ receptors relative to brain cells (Tyndale et al., 1994). $GABA_A$ receptors of IMR-32 cells have not been electrophysiologically examined; however, radioligand binding data suggest that like RINm5F, βTC3, and GT1-7 cells, the neuroblastoma cell line expresses fewer receptors than do primary brain cells (Noble et al., 1993).

Similarities between the properties of $GABA_A$ receptors of the GABA responsive cell lines may be due to the expression of similar $GABA_A$ receptor subunits. Indeed, the cell lines have certain $GABA_A$ receptor transcripts and proteins in common. GT1-7 (Hales et al., 1992; Favit et al., 1993; Kirkness and Fraser, 1993; Kim, personal communication), RINm5F and βTC3 (Tyndale et al., 1994) cells have in common, detectable levels of α3, β3, and δ $GABA_A$ receptor subunit mRNAs. In addition, IMR-32 cells have α3 subunit protein identified by immunoblotting. The presence of β3 and δ $GABA_A$ receptor transcripts or proteins in IMR-32 cells has not been investigated. However, the available data suggest that RINm5F, βTC3, GT1-7, and IMR-32 cell lines have functional $GABA_A$ receptors with similar properties and probably some common receptor subunits. Further studies will be necessary in order to identify which additional common subunits are expressed by these cell lines.

$GABA_A$ receptors in the endocrine pancreas

RINm5F cells, derived from an x-ray induced rat islet tumor, have been extensively studied in investigations of β-cell function (Gazdar et al., 1980; Eddlestone et al., 1989). However, in addition to insulin these cells synthesize and secrete the pancreatic α-cell hormone glucagon (Barreto et al., 1989) and low levels of the δ-cell hormone somatostatin (Gazdar et al., 1980). There have been no reports of β-cells expressing $GABA_A$ receptors. In contrast, α-cells respond electrophysiologically to GABA, and both α- and δ-cells bind antibodies to the β subunit of the $GABA_A$ receptor (Rorsman et al., 1989). Hence, RINm5F cells secrete glucagon, somatostatin and insulin, and express $GABA_A$ receptors.

$GABA_A$ receptors of RINm5F cells have properties similar to those of embryonic neurons. The receptors are sensitive to Zn^{2+}, but insensitive to diazepam indicating a lack of γ subunits. $GABA_A$ receptors of embryonic neurons are more sensitive to Zn^{2+} than are receptors of adult neurons (Smart and Constanti, 1990), suggesting that they are also deficient in γ subunits (Draguhn et al., 1990; Smart et al., 1991). In contrast to RINm5F cells, GABA-activated currents recorded from primary cultured guinea pig pancreatic α-cells are "approximately doubled" by diazepam (Rorsmann et al., 1989). This indicates that unlike tumoral RINm5F cells, α-cells express $GABA_A$ receptors containing γ subunits. $GABA_A$ receptors of δ cells have not been characterized and it is not known whether they contain γ subunits. However, our demonstration of Zn^{2+}-sensitive, diazepam-insensitive $GABA_A$ receptors in RINm5F cells, supports the hypothesis that these cells represent clonal pancreatic cells of an immature phenotype.

In addition to $GABA_A$ receptor Cl^- channels, RINm5F cells also express other ion channels normally found in β-cells. For example, RINm5F cells have ATP-sensitive K^+ channels (Cook and Hales, 1984; Eddlestone et al., 1989) and Ca^{2+}- and voltage-activated K^+ [K(Ca,V)] channels (Cook et al., 1984; Eddlestone et al., 1989). Interestingly, K(Ca,V) channels of RINm5F cells, in common with those of neonatal rat β-cells (Cook et al., 1984), have a relatively low sensitivity to Ca^{2+}. This observation is consistent with RINm5F cells being of an immature phenotype (Eddlestone et al., 1989). Taken together, our data characterizing functional $GABA_A$ receptors, combined with results of previous electrophysiological and secretion studies, suggest that RINm5F cells may be immature tumoral progenitor cells of the endocrine pancreas, with α-, β-, and δ-cell properties.

The role of GABA in the endocrine pancreas is poorly understood. Pancreatic β-cells have the GABA synthesizing enzyme glutamate decarboxylase (Sorenson et al., 1991) and small synaptic like microvessicles, with GABA-uptake pumps (Thomas-Reetz et al., 1993), which may mediate GABA release from these cells. Glucose-induced insulin secretion from β-cells is associated with an inhibition of glucagon secretion from α-cells. The latter is blocked when isolated islets of Langerhans are incubated with the $GABA_A$ receptor antagonist bicuculline (Rorsman et al., 1989). This observation led to the suggestion that glucose-induced insulin secretion was accompanied by an increase in the release of GABA from β-cells. GABA binds to receptors on α-cells, perhaps causing hyperpolarization and thus

reducing their secretion of glucagon (Rorsman et al., 1989). While this is an attractive hypothesis for the role of GABA in the islet, whether glucose enhances GABA release from β-cells is the subject of debate (Rorsman et al., 1989; Sorenson et al., 1991). In addition, although some studies have shown that GABA inhibits glucagon secretion from α-cells (Rorsman et al., 1989), and the secretion of somatostatin from islet δ-cells, these observations are controversial (Sorenson et al., 1991). It has been suggested that GABA may be acting purely in a metabolic context within β-cells (Sorenson et al., 1991). With the recent demonstrations of the importance of autoimmunity to the GABA-producing enzyme GAD in the onset of insulin-dependent diabetes mellitus in nonobese diabetic mice (Kaufman et al., 1993; Tisch et al., 1993), there is renewed interest in the physiological role of GABA in the endocrine pancreas. Our demonstration that RINm5F cells express functional receptors provides a system in which to study the characteristics and regulation of expression of pancreatic $GABA_A$ receptors.

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