cell survival and homeostatic proliferation have suggested that low-affinity or antagonist peptides are responsible (*11*–*13*). It is intriguing, therefore, that homeostatic proliferation of T cells is more lck-sensitive than T cell survival. Although it has previously been assumed that the same signals mediate survival and homeostasis, the data from this study suggest otherwise. We found that T cells survived without lck expression, but that homeostatic proliferation of naïve T cells in T cell–deficient hosts occurred only when lck transgene expression was maintained. This suggests that different signals are required for survival and proliferation, perhaps requiring distinct ligands or different growth and survival factors.

In conclusion, our data suggest that a hierarchy of signals govern T cell behavior. Activation of T cells in response to strong agonist signals is highly lck-dependent, as indicated by the poor T cell responses to CD3 stimulation in the absence of lck (Fig. 2D) and the phenotype of lck-deficient mice (*15*). Homeostatic proliferation may be driven by weak or partial agonist signals that continue to depend on lck activity but that differ from strong agonist signals in that they do not necessarily result in progression of cells to the memory pool (*25*, *26*). In contrast, T cell survival signals can be provided by antagonist signals alone that are relatively lck-insensitive (*16*, *27*) and may instead depend on other src family members such as fyn (*28*, *29*). Although all of these signals may promote survival, only those that activate lck mediate proliferative responses.

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17. That lck is implicated in lineage commitment decisions in the thymus has been demonstrated by a number of recent studies (*16*, *30*). In Lck1/rtTA-C/lck^{neg} mice fed dox, levels of the transgene exceed those found in WT mice, conditions favoring the development of CD4$^+$ over CD8$^+$ T cells. Consequently, the periphery of these mice contains fewer CD8$^+$ T cells than the periphery of WT mice, but both CD4$^+$ and CD8$^+$ cells were shown to be of the appropriate MHC restriction (*16*).
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32. Splenic T cells were purified from mice by means of mouse T cell–enrichment columns (R&D Systems) and RNA extracted with RNAzol B (Tel-Test). Lck1 transgene transcripts were detected by RT-PCR with primers that specifically amplify a 461–base pair product spanning the mouse Lck and SV40 polyadenylation signal of the transgene construct: forward primer, CAGTACCAGCCCCAGCCTTGATAG (mouse Lck); reverse primer, GCAGTCCAGCTTTTCCTTTGTGG [SV40 poly(A)]. PCRs were performed in the presence and absence of RT to exclude genomic DNA contamination of RNA, and PCR for HPRT (hypoxanthine-guanine phosphoribosyltransferase) mRNA was performed to ensure that equivalent amounts of total RNA were compared.

33. We thank T. Norton, P. Travel, and K. Williams for conscientious care of mice and G. Stockinger and D. Kioussis for helpful discussions and comments on the manuscript. B.S. is supported by a grant from the Leukemia Research Fund. Additional funding for this work was provided by the Medical Research Council.

19 June 2000; accepted 31 August 2000

Molecular and Neuronal Substrate for the Selective Attenuation of Anxiety

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Benzodiazepine tranquilizers are used in the treatment of anxiety disorders. To identify the molecular and neuronal target mediating the anxiolytic action of benzodiazepines, we generated and analyzed two mouse lines in which the α2 or α3 $GABA_A$ (γ-aminobutyric acid type A) receptors, respectively, were rendered insensitive to diazepam by a knock-in point mutation. The anxiolytic action of diazepam was absent in mice with the α2(H101R) point mutation but present in mice with the α3(H126R) point mutation. These findings indicate that the anxiolytic effect of benzodiazepine drugs is mediated by α2 $GABA_A$ receptors, which are largely expressed in the limbic system, but not by α3 $GABA_A$ receptors, which predominate in the reticular activating system.

Excessive or inappropriate anxiety can be controlled by enhancing inhibitory synaptic neurotransmission mediated by GABA (GABAergic inhibitory neurotransmission) using clinically effective benzodiazepine drugs (*1*). However, to date it has not been possible to identify the one or more $GABA_A$ receptor subtypes that mediate the attenuation of anxiety. Four types of diazepam-sensitive $GABA_A$ receptors can be distinguished on the basis of the presence of α1, α2, α3, or α5 subunits. These receptors can be rendered insensitive to diazepam in vitro by replacing a conserved histidine residue by arginine in the drug binding site (*2, 3*). Introduction of the respective point mutation into mouse lines enables the pharmacological profile of benzodiazepine drugs to be attributed to defined receptor subtypes. Using this approach, we have attributed the sedative and amnesic prop-

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erties of diazepam, but not its anxiolytic action, to α1 $GABA_A$ receptors (*4, 5*). We hypothesized that the anxiolytic action of benzodiazepine drugs might be mediated by α2 or α3 $GABA_A$ receptors on the basis of their distinct neuroanatomical expression patterns. Whereas α2 $GABA_A$ receptors are preponderant in areas of the limbic system as well as in the cerebral cortex and striatum, α3 $GABA_A$ receptors are selectively expressed in noradrenergic and serotonergic neurons of the brainstem reticular formation, the basal forebrain cholinergic neurons, and GABAergic neurons in the reticular nucleus of the thalamus (*6*).

To distinguish the pharmacology of the α2 receptor subtype from that of the α3 subtype, we generated two mouse lines in which a point mutation was introduced into homologous positions of the α2 subunit gene (Fig. 1) and the α3 subunit gene (*7*), rendering the respective receptors insensitive to diazepam. [The point mutations were $His^{101} \rightarrow Arg$ and $His^{126} \rightarrow Arg$, respectively, hence the mouse lines are denoted α2(H101R) and α3(H126R).] The mutants did not display an overt distinctive phenotype, bred normally, and expressed all subunits tested (α1, α2, α3, β2/3, and γ2) at normal levels (Fig. 2A) and with unaltered distribution. Ro15-4513 is an inverse agonist at the benzodiazepine binding site and binds to both diazepam-sensitive and diazepam-insensitive $GABA_A$ receptors. The proportion of diazepam-insensitive [^{3}H]Ro15-4513 binding sites was increased from 5% in wild-type mice to 17% in α2(H101R) mice and to 11% in α3(H126R) mice [α2 wild-type controls: maximum number of binding sites B_{max} = 0.08 ± 0.02 pmol/mg protein, dissociation constant K_d = 4.3 ± 0.8 nM (n = 3); α2(H101R) mice: B_{max} = 0.26 ± 0.01 pmol/mg protein, K_d = 8.0 ± 1.3 nM (n = 3); α3 wild-type controls: B_{max} = 0.06 ± 0.01 pmol/mg protein, K_d = 4.9 ± 2.1 nM (n = 3); and α3(H126R) mice: B_{max} = 0.11 ± 0.02 pmol/mg protein, K_d = 6.5 ± 1.6 nM (n = 3)]. In line with the known distribution of the α2 subunit (*6*), novel diazepam-insensitive sites in α2(H101R) mice were visualized in all regions expressing α2 $GABA_A$ receptors, as shown autoradiographically in parasagittal brain sections using 10 nM [^{3}H]Ro15-4513 in the presence of 100 μM diazepam (Fig. 2B). Similarly, in α3(H126R) mice, the novel diazepam-insensitive [^{3}H]Ro15-4513 binding sites displayed a distribution corresponding to that of the α3 subunit (Fig. 2B) (*6*). After immunoprecipitation with α2 or α3 subunit–specific antisera, [^{3}H]Ro15-4513 binding revealed a decrease of more than three orders of magnitude in the affinity of diazepam to the α2 and α3 subunits from the respective mutant mice.

In cultured hippocampal pyramidal cells (*8*), the electrophysiological response to GABA (3 μM) was indistinguishable between cells from wild-type and α2(H101R) mice (Fig. 2C). However, the potentiation by diazepam (1 μM) was reduced in cells from α2(H101R) mice relative to cells from wild-type mice [17.6 ± 4.5% (n = 29) versus 48.1 ± 7.9% (n = 18), P = 0.001] (Fig. 2C); the remaining potentiation presumably can be attributed to $GABA_A$ receptors other than α2. The inverse agonistic action of Ro15-4513 (1 μM) in wild-type cells was converted into an agonistic response in cells derived from α2(H101R) mice [–39 ± 5.2% (n = 13) versus 11.7 ± 7.5% (n = 23), P = 0.003] (Fig. 2C). This is consistent with the switch in efficacy of Ro15-4513 from inverse agonism to agonism, as shown for recombinant α2(H101R)β3γ2 receptors expressed in HEK-293 cells (*3*).

The pharmacological importance of the

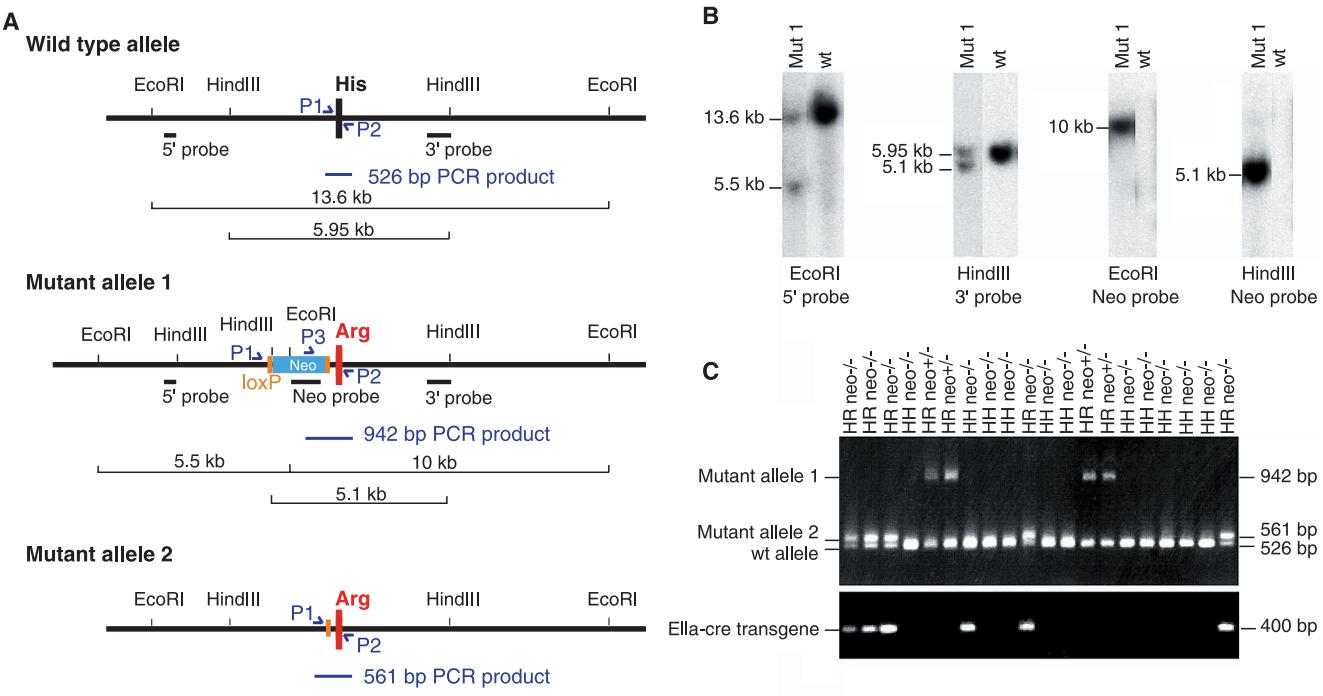

Fig. 1. Targeting of the α2 subunit $GABA_A$ receptor gene. (**A**) Structure of wild-type and mutant alleles. Mutant allele 1 is obtained after gene targeting in mouse embryonic stem cells and introduced into the mouse germ line; breeding of these mice to EIIa-cre mice (*16*) results in excision of the neomycin resistance cassette (mutant allele 2). The 5′ and 3′ probes flanking the targeting vector are drawn as solid bars. His and Arg denote codons for histidine and arginine, respectively, at position 101 in exon 4. Polymerase chain reaction (PCR) primers P1, P2, and P3 are indicated. (**B**) Southern blot analysis of wild-type (wt) allele and mutant allele 1 (Mut1) in embryonic stem cells. (**C**) Genotyping offspring from a cross of a chimera and a mouse hemizygous for the EIIa-cre transgene. Top panel: Primers P1, P2, and P3 provide specific amplification products for each allele. Bottom panel: PCR primers UR26 and UR36 amplify the *cre* transgene. (**D**) Verification of the α2(H101R) point mutation by automated DNA sequencing.

mutated α2 and α3 $GABA_A$ receptors was assessed by comparing the diazepam-induced behavior of α2(H101R) and α3(H126R) mice with that of wild-type mice (*9*). When tested in a dose-dependent manner, the sedative, motor-impairing, and anticonvulsant actions of diazepam (*10*) were not impaired in either α2(H101R) mice or α3(H126R) mice relative to wild-type mice (Fig. 3).

The anxiolytic-like action of diazepam in α2(H101R) and α3(H126R) mice was investigated in the light-dark choice test (*11*) and the elevated plus-maze test (*12*). In the light-dark choice test, the α2(H101R) mice did not show the behavioral disinhibition by diazepam that was apparent in wild-type mice. Diazepam up to 2 mg/kg body weight did not increase the time spent in the lit area in α2(H101R) mice relative to wild-type mice ($P < 0.05$ versus vehicle) (Fig. 4A). This effect was not due to a motor deficit in α2(H101R) mice, because no behavioral differences in the dark area were observed between wild-type and α2(H101R) mice under either vehicle or diazepam treatment. Furthermore, α2(H101R) mice retained the ability to display an anxiolytic-like response to ligands acting at $GABA_A$ receptor sites other than the benzodiazepine site. Sodium phenobarbital (15 mg/kg subcutaneously) induced a behavioral disinhibition in the light/dark choice test in α2(H101R) mice similar to that seen in wild-type mice [time in lit area, wild-type: vehicle, 70.5 ± 10.5 s, phenobarbital, 111.75 ± 10.0 s; α2(H101R): vehicle, 75.3 ± 11.5 s, phenobarbital, 110.9 ± 9.6 s; $F(1,24) = 13.44$, $P < 0.01$, $n = 6$ to 8].

The absence of an anxiolytic-like effect of diazepam in α2(H101R) mice was confirmed in the elevated plus-maze test. In wild-type mice, diazepam facilitated the exploratory behavior by increasing both the amount of time spent ($P < 0.01$ versus vehicle) and the number of entries in the open arms ($P < 0.05$). In contrast, in α2(H101R) mice, diazepam failed to increase both parameters of exploratory behavior (Fig. 4, B and C). Again the failure was not due to motor impairment, because the motor activity in the enclosed arms was similar in α2(H101R) and wild-type mice irrespective of the treatment.

The potential contribution of α3 $GABA_A$ receptors to the anxiolytic-like activity of diazepam was examined in α3(H126R) mice. Both α3(H126R) and wild-type mice displayed similar dose-dependent anxiolytic-like

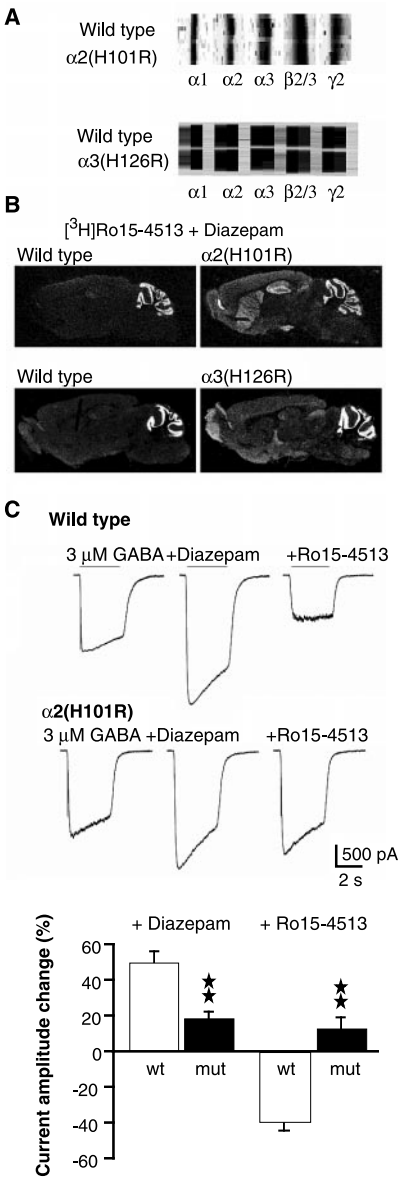

Fig. 2. Molecular characteristics of $GABA_A$ receptors in α2(H101R) and α3(H126R) mice. (**A**) Western blots of whole brain membranes from wild-type, α2(H101R), and α3(H126R) mice using antisera recognizing the α1, α2, α3, β2/3, and γ2 subunits. (**B**) Receptor autoradiography of diazepam-insensitive sites in wild-type, α2(H101R), and α3(H126R) brains. Parasagittal sections were incubated with 10 nM [3H]Ro15-4513 in the presence of 100 μM diazepam to reveal the diazepam-insensitive [3H]Ro15-4513 binding sites. In wild-type mice, diazepam-insensitive [3H]Ro15-4513 binding is due to α4 and α6 $GABA_A$ receptors. (**C**) GABA responses in cultured hippocampal pyramidal cells from α2(H101R) mice. The holding potential in the patch-clamp analysis was −60 mV; the chloride concentration was symmetrical. GABA was applied for 5 s. Hippocampal neurons from embryonic day 16.5 embryos were cultured for 10 to 14 days. **, $P < 0.001$ (Student's *t* test).

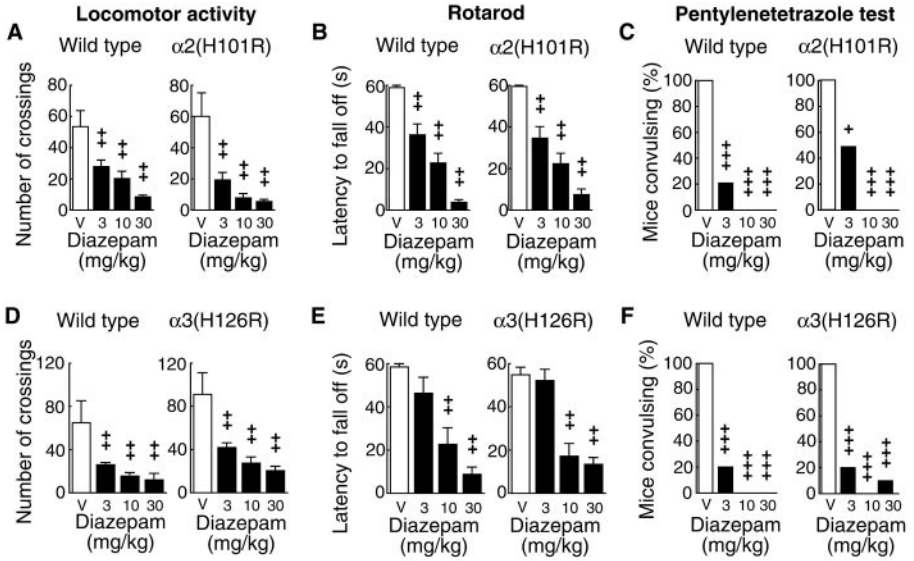

Fig. 3. Behavioral assessment of the sedative, motor-impairing, and anticonvulsant properties of diazepam in α2(H101R) and α3(H126R) mice relative to wild-type mice. (**A**) Dose-dependent inhibition of locomotor activity in wild-type and α2(H101R) mice [$F(3,71) = 19.31$, $P < 0.001$, $n = 9$ or 10 mice per group]. (**B**) Dose-dependent decrease in the latency to fall off the rotating rod (fixed 2 rpm) in wild-type and α2(H101R) mice [$F(3,153) = 71.01$, $P < 0.001$, $n = 20$ or 21 mice per group]. (**C**) Dose-dependent decrease of the percentage of mice developing tonic convulsions in wild-type ($\chi^2 = 32.38$, $P < 0.001$, $n = 10$ mice per group) and α2(H101R) mice ($\chi^2 = 28.13$, $P < 0.001$, $n = 10$ mice per group). (**D**) Dose-dependent inhibition of locomotor activity in wild-type and α3(H126R) mice [$F(3,64) = 14.70$, $P < 0.001$, $n = 8$ to 10 mice per group]. (**E**) Dose-dependent decrease in the latency to fall off the rotating rod (fixed 2 rpm) in wild-type and α3(H126R) mice [$F(3,64) = 36.78$, $P < 0.001$, $n = 8$ to 10 mice per group]. (**F**) Dose-dependent decrease of the percentage of mice developing tonic convulsions in wild-type ($\chi^2 = 32.38$, $P < 0.001$, $n = 10$ mice per group) and α3(H126R) mice ($\chi^2 = 28.60$, $P < 0.001$, $n = 10$ mice per group). Results are given as means ± SEM. +, $P < 0.05$; ++, $P < 0.01$; +++, $P < 0.001$ (Dunnett's post hoc comparisons or Fisher's exact tests). V, vehicle. The rotarod and pentylenetetrazole convulsion tests were performed according to Bonetti *et al.* (*10*). Locomotor activity was automatically recorded for 30 min. Mice were treated with either vehicle or diazepam (3, 10, and 30 mg/kg orally) 30 min before testing.

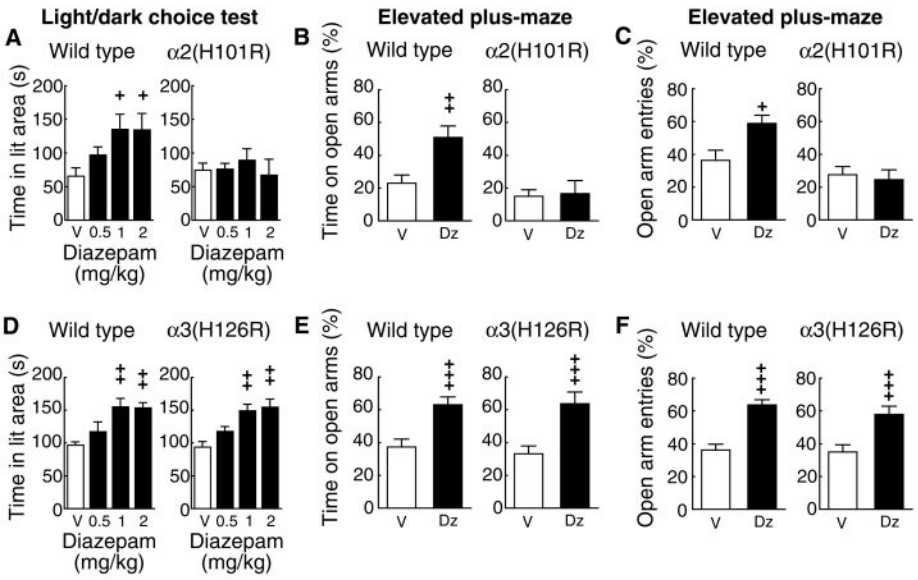

Fig. 4. Behavioral assessment of anxiolytic-like action of diazepam in α2(H101R) and α3(H126R) mice relative to wild-type mice. (**A**) Light/dark choice test. Diazepam dose-dependently increased the time spent in the lit area in wild-type mice [$F(3,36) = 3.14$, $P < 0.05$] but not in α2(H101R) mice [$F(3,36) = 0.32$, not significant] ($n = 10$ mice per group). (**B** and **C**) Elevated plus-maze. Diazepam (2 mg/kg) increased the percentage of time spent on the open arms and the number of entries on the open arms in wild-type mice ($P < 0.01$ and $P < 0.05$ versus vehicle) but not in α2(H101R) mice [$F(1,32) = 4.31$ and $F(1,32) = 4.76$, $P < 0.05$, respectively] ($n = 8$ to 10 mice per group). (**D**) Light/dark choice test. Both wild-type and α3(H126R) mice displayed a dose-dependent increase in the time spent in the lit area [$F(1,70) = 14.74$, $P < 0.001$, $n = 9$ or 10 mice per group]. (**E** and **F**) Elevated plus-maze. Diazepam (2 mg/kg) increased the percentage of time spent on the open arms and the number of entries on the open arms to the same extent in wild-type and α3(H126R) mice [$F(1,36) = 26.52$ and $F(1,36) = 37.31$, $P < 0.001$, respectively] ($n = 10$ mice per group). Results are given as means ± SEM. +, $P < 0.05$; ++, $P < 0.01$; +++, $P < 0.001$ (Dunnett's or Fisher's pairwise post hoc comparisons or Fisher's exact tests). V, vehicle; Dz, diazepam. The light-dark choice test was carried out as described (*11*) with an illumination of 500 lux. Mice were given vehicle or increasing doses of diazepam (0.5, 1, and 2 mg/kg orally). The elevated plus-maze was performed according to Lister (*12*) under an indirect dim-light illumination (< 10 lux). Vehicle or diazepam were administered 30 min before testing.

responses to diazepam in the light/dark choice test ($P < 0.01$ versus vehicle) (Fig. 4D) and in the elevated plus-maze ($P < 0.001$ versus vehicle) (Fig. 4, E and F). These results indicate that the anxiolytic action of diazepam in wild-type mice does not involve interaction with α3 $GABA_A$ receptors.

The anxiolytic-like action of diazepam is selectively mediated by the enhancement of GABAergic transmission in a population of neurons expressing the α2 $GABA_A$ receptors, which represent only 15% of all diazepam-sensitive $GABA_A$ receptors (*13*). The α2 $GABA_A$ receptor–expressing cells in the cerebral cortex and hippocampus include pyramidal cells that display very high densities of α2 $GABA_A$ receptors on the axon initial segment, presumably controlling the output of these principal neurons (*14, 15*). Our findings indicate that the α2 $GABA_A$ receptors are highly specific targets for the development of future selective anxiolytic drugs.

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8. Cultured hippocampal pyramidal cells were chosen as a model system to confirm that the pharmacological properties of recombinant mutant α2 $GABA_A$ receptors can also be demonstrated for $GABA_A$ receptors in mutant mice.
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17. We thank Y. Lang for blastocyst injection; P. Fakitsas and C. Michel for performing immunoblot, autoradiography, and ligand binding experiments; C. Sidler for hippocampal cell culture; D. Blaser, H. Pochetti, and G. Schmid for animal care; H. Westphal for EIIa-cre mice; H. Hengartner for E14 embryonic stem cells; and E. M. Simpson for mEMS32 embryonic stem cells. Supported by a grant from the Swiss National Science Foundation.

9 June 2000; accepted 10 August 2000

Function of GATA Transcription Factors in Preadipocyte-Adipocyte Transition

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Genes that control the early stages of adipogenesis remain largely unknown. Here, we show that murine GATA-2 and GATA-3 are specifically expressed in white adipocyte precursors and that their down-regulation sets the stage for terminal differentiation. Constitutive GATA-2 and GATA-3 expression suppressed adipocyte differentiation and trapped cells at the preadipocyte stage. This effect is mediated, at least in part, through the direct suppression of peroxisome proliferator–activated receptor γ. GATA-3–deficient embryonic stem cells exhibit an enhanced capacity to differentiate into adipocytes, and defective GATA-2 and GATA-3 expression is associated with obesity. Thus, GATA-2 and GATA-3 regulate adipocyte differentiation through molecular control of the preadipocyte-adipocyte transition.

In vertebrates, adipose tissue is critical for energy storage and release, as well as for endocrine homeostasis (*1, 2*). The two general classes of fat cells in mammals, brown and white, have different functions. White adipose tissue (WAT) stores excess energy in the form of triglyceride and releases free fatty acids during caloric deficiency. Brown adipose tissue (BAT), on the other hand, can dissipate energy through thermogenesis. The