A Simple Genetic Basis for a Complex Psychological Trait in Laboratory Mice

Jonathan Flint,* Robin Corley, John C. DeFries, David W. Fulker, Jeffrey A. Gray, Stacey Miller, Allan C. Collins

Psychological traits are commonly inferred from covariation in sets of behavioral measures that otherwise appear to have little in common. Emotionality in mice is such a trait, defined here by covariation in activity and defecation in a novel environment and emergence into the open arms of an elevated plus maze. Behavioral and quantitative trait analyses were conducted on four measures obtained from 879 mice from an F_2 intercross. Three loci, on murine chromosomes 1, 12, and 15, were mapped that influence emotionality. This trait, inferred from studies of strain, sex, and individual differences in rodents, may be related to human susceptibility to anxiety or neuroticism.

A genetic contribution to psychological traits and psychiatric disorder is not in doubt, but the nature of that contribution is still poorly understood (*1*). Where quantitative variation in such traits is known to exist, its genetic basis might be accessible to the linkage mapping approaches that have been successful in the analysis of physiological traits with a multifactorial etiology (*2*), but setbacks in attempts to map the susceptibility loci for human psychiatric disorders have suggested that psychological traits may be determined by a large number of genes each of small effect (rather than by a few major genes) such that their operation would be undetectable by most quantitative trait loci (QTL) linkage strategies (*3*).

Furthermore, it is also not clear which measures of a psychological trait are amenable to linkage analysis. Genetic mapping of human psychiatric disorders has generally used a dichotomous classification (affected versus nonaffected). Although collection of appropriate samples may lead to the chromosomal mapping of dichotomous traits, recent success in mapping a susceptibility locus for reading disability and the loci responsible for drug preference in mice indicates that continuous measures may be more appropriate (*4*). However, a single behavioral or psychiatric measure is likely to include a number of traits not directly related to the psychological trait under study, so that a mapping experiment could result in the localization of QTLs that are not of interest. An alternative approach would be to use a psychological trait defined by the covariation of a set of measures, but so far multiple measures have not been used in mapping experiments of behavioral traits. Here we report the use of such an approach to map QTLs underlying measures of emotionality in mice. Calvin Hall first applied the term "emotionality" to rodent behavior defined by a specific set of behavioral measures (*5*). It has also been termed reactivity (*6*), but despite its name, emotionality does not include all aspects of emotion in the broad sense of the term (*7*).

Rodent emotionality has long been measured by behavior in novel environments such as the open-field apparatus, a brightly lit white arena which is an unwelcoming and potentially frightening environment (*5*). Low activity and high defecation scores in the apparatus have been used to define emotionality, and selection experiments show that these measures are genetically correlated. Rats selected for differences in defecation in the open field differ also in activity scores in the apparatus (*6*), whereas mice selected for differences in activity show differences in defecation (*8, 9*). In both species, animals with high defecation scores are inactive, and vice versa. Through the inclusion of control and replicate lines, it has been shown that this inverse genetic correlation is not a chance association due to genetic drift occurring in the selection experiment; rather, it is evidence for the existence of a psychological trait. Moreover, known physiological mechanisms cannot explain the covariation of defecation with open-field activity (OFA): OFA involves primarily motor activity, whereas defecation is an autonomic response that does not require motor performance.

The genetic selection experiments support the notion that emotionality (defined as the phenotypic correlation between OFA and defecation) involves the same brain system or systems, and a wide range of pharmacological, brain lesion, and brain stimulation experiments indicate which are the most likely brain structures involved (*7, 10*). Chromosomal mapping of correlated behavioral measures that define emotionality would provide a novel approach to the validation of the psychological trait, which may be related to human susceptibility to anxiety (*7, 10*).

Inbred mouse strains derived from animals selected for both high and low OFA scores presented an opportunity to map emotionality. Furthermore, the selection experiment provided estimates of the realized heritability of OFA (0.26) and defecation (0.11) and of the genetic correlation between the two traits (−0.86) (*9*), which allowed us to infer from the QTL analysis the likely number of genes and the direction of their effects.

Our experiment had two stages: stage 1 was to detect QTLs for OFA, and stage 2 was to determine whether QTLs identified for OFA were common to other measures whose covariation with OFA indicates emotionality. We chose measures with known phenotypic correlations for which there was either genetic or other evidence that they involve the same brain systems (*7, 10*). Because of the large number of animals that needed to be tested, we also chose measures that were simple and quick to administer.

We measured OFA and defecation and two additional measures: activity in a Y maze and entry into the open and closed arms of an elevated plus maze (EPM) (*11, 12*). The Y maze, like the open-field arena, is a novel, potentially frightening environment for the mouse; it differs in that it is completely enclosed and is dark within. The EPM is a four-armed runway arranged in a cross shape raised 30 cm above the ground, two arms of which are enclosed. It has been shown that exploration of the open arms is more anxiogenic than exploration of the closed arms (*11*).

On the basis of previous work (*7*) we predicted that genes determining low OFA would determine low activity in other novel environments (in this case the Y maze), high open-field defecation, and reduced entries into the open arms of the EPM. Entry into the closed arms of the EPM does not correlate with the other measures (it is a measure of activity, not of emotionality) and was included as a negative control (*12*).

From 879 F_2 intercross progeny, sequentially tested in the open field (for activity and defecation), Y maze, and EPM, we initially genotyped 96 mice with the highest and 96 with the lowest OFA scores (representing the extreme 10%) by means of 84 markers covering the mouse genome (*13*). Genotype data were entered into the MAPMAKER-QTL analysis program (*14*).

A LOD (logarithm of the likelihood ratio for linkage) threshold of 3.23 was used for the detection of QTLs in the initial genome scan of 192 animals. It has been recommended that a LOD threshold of 4.3

J. Flint, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK.
R. Corley, J. C. DeFries, D. W. Fulker, S. Miller, A. C. Collins, Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309, USA.
J. A. Gray, Institute of Psychiatry, London SE5 8AF, UK.
*To whom correspondence should be addressed.

Table 1. Quantitative trait loci for OFA. The LOD score is the logarithm of the likelihood for the presence of a QTL. The value *F* is from a multiple regression analysis with two degrees of freedom for additive and dominance effects. *P* is the probability of the *F* test result. The particular chromosome on which a QTL located is specified by the number after the "D" in the marker name. cM, centimorgan.

Map position	LOD score	*F*	*P*
D1Mit150 + 0 cM	11.9	22.9	<0.00001
D4Mit81 + 0 cM	7.0	13.0	<0.00001
D12Mit47 + 6 cM	3.7	4.3	0.015
D15Mit63 + 3 cM	7.2	5.4	0.005
D17Mit24 + 0 cM	4.0	3.0	0.054
D18Mit67 + 10 cM	3.9	2.6	0.076

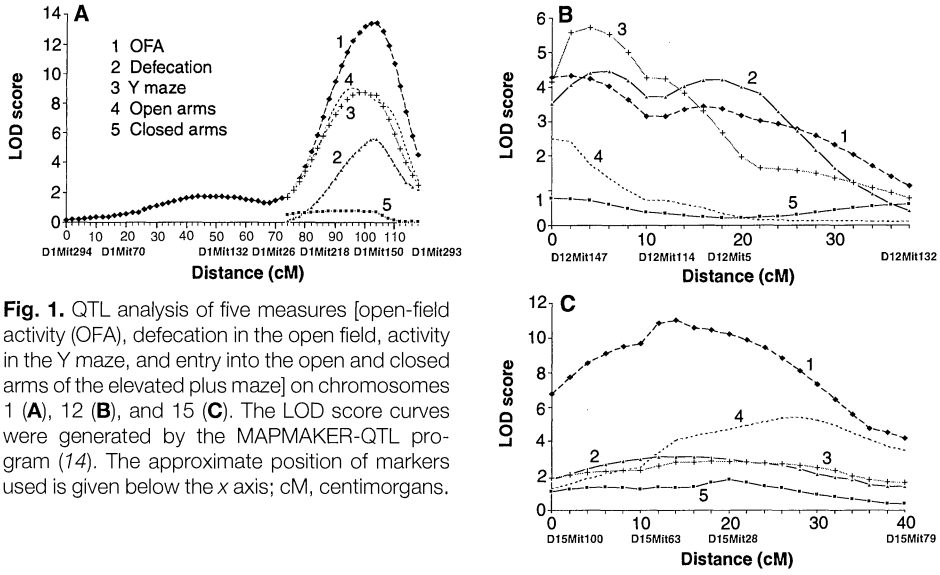

Fig. 1. QTL analysis of five measures [open-field activity (OFA), defecation in the open field, activity in the Y maze, and entry into the open and closed arms of the elevated plus maze] on chromosomes 1 (**A**), 12 (**B**), and 15 (**C**). The LOD score curves were generated by the MAPMAKER-QTL program (*14*). The approximate position of markers used is given below the *x* axis; cM, centimorgans.

be adopted for a whole-genome search because a single LOD exceeding that threshold is likely to arise by chance with a probability of less than 1 in 20 (*15*). However, the probability of a number (n) of unlinked QTLs exceeding the 4.3 threshold by chance is 0.05^n. Because we expected to detect multiple QTLs, a lower threshold was used to avoid false negative results. We therefore derived a LOD threshold of 3.23 by dividing a 0.05 probability by the number of tests performed (84 loci) and calculating the LOD equivalent of this probability from a χ^2 distribution with two degrees of freedom (because MAPMAKER-QTL assesses both additive and dominant models). Six loci were detected that exceeded this threshold (Table 1). In addition we used a stepwise multiple regression test with two degrees of freedom for additive and dominance effects at the marker closest to each of these putative QTLs. The results are shown in Table 1.

We next investigated whether the six QTLs underlying OFA could also influence the correlated behaviors of defecation, activity in the Y maze, and entry into the open arms of the EPM. By selecting 192 more mice we were able to genotype enough animals to include the highest and lowest 10% of all measures. Therefore, a total of 384 animals were genotyped with three markers for each of the six chromosomal regions indicated by the OFA analysis (Table 2).

The second analysis attempts to determine the likelihood that the six QTLs discovered for OFA could also be QTLs for three other behavioral measures. This does not involve mapping QTLs for other measures throughout the genome and asking whether any of these coincided with the QTLs already mapped, so the previously derived LOD threshold of 3.23 is not applicable. Instead, we used a LOD threshold of 2.7 on the grounds that because we had tested for the presence of six QTLs, the appropriate χ^2 is 9.55 (corresponding to a level of 0.05 divided by six, where six is the number of putative QTLs). We then added $\log(k)$ to the LOD derived from the χ^2 value, where k is the number of measures (*16*). We expect this to be a conservative approach.

Table 2 shows that loci on chromosomes 1, 12, and 15 were identified with high LOD scores for the four correlated measures. On chromosome 12, a QTL for the open-arm EPM data was mapped with a LOD of only 2.4, but QTLs for the other measures exceeded our threshold value of 2.7 (Fig. 1). The double peak on chromosome 12 suggested the presence of more than one QTL, but we found no evidence for this when MAPMAKER-QTL was used to fix one QTL (on chromosome 12) and search for another on the same chromosome. Similar analysis on chromosome 15 data also gave no evidence for multiple QTLs. As expected, no QTLs were detected for entry into the closed arms of the EPM, confirming that the correlation between

Table 2. Quantitative trait loci for four behavioral measures. Chr, chromosome; LOD, logarithm of the likelihood for the presence of a QTL; Weight, the additive component contributed by an allele from the H_1 strain; %Var, the percentage of phenotypic variance explained by the QTL; ΔR^2, the change in multiple R^2 using the two degrees of freedom test of additive and dominance effects; *F*, two degrees of freedom test for additive and dominance effects using multiple regression analysis; *P*, probability of obtaining the *F* test result.

Chr	LOD	Weight	%Var	ΔR^2	*F*	*P*	LOD	Weight	%Var	ΔR^2	*F*	*P*
	Open-field activity						*Defecation*					
1	13.4	1.11	9.2	12.6	36.79	<0.00001	5.6	−0.24	5.3	6.1	12.93	<0.00001
4	6.2	0.87	4.4	6.1	17.76	<0.00001	1.1	−0.11	1.1	1.1	2.38	0.0938
12	4.3	0.71	3.4	3.0	8.86	0.0002	4.5	−0.22	5.2	3.4	7.37	0.0007
15	11.0	1.19	8.1	5.9	17.19	<0.00001	3.1	−0.21	3.0	1.9	4.15	0.0166
17	3.5	0.68	2.4	1.8	5.12	0.0064	0.8	−0.09	0.8	0.9	1.83	0.1617
18	4.0	0.45	4.2	1.5	4.37	0.0133	0.7	−0.03	0.9	0.2	0.49	0.6118
	Y maze						*Elevated plus maze*					
1	8.7	0.34	8.4	8.1	17.10	<0.00001	9.0	0.38	21.0	6.9	13.40	<0.00001
4	2.1	0.17	1.6	1.5	3.08	0.0472	1.2	0.12	1.4	1.4	2.74	0.0662
12	5.7	0.33	5.1	4.1	8.57	0.0002	2.4	0.15	3.1	1.7	3.20	0.0422
15	2.8	0.21	2.8	1.0	2.18	0.1141	5.4	0.33	14.0	1.8	3.48	0.0319
17	0.4	0.06	0.4	0.0	0.08	0.9268	1.5	0.02	2.2	0.8	1.52	0.2196
18	1.3	0.15	1.0	0.8	1.75	0.1750	1.3	0.02	2.6	0.9	1.67	0.1901

entry into the open arms and OFA is not mediated merely by activity (Fig. 1).

Table 2 also shows the amount of phenotypic variance explained by each QTL for the four behavioral measures. Previous work (9) estimated the realized heritability of OFA to be 26% and of defecation to be 11%, although heritability in the F_2 generation may be higher than these figures suggest if there is linkage disequilibrium between QTLs on the same chromosome. Nevertheless, because the six QTLs for OFA account for 31% and the three QTLs for defecation account for 14% of the phenotypic variance, it is likely that we have identified all major contributors to the genetic variance of these measures. The congruence between realized heritability and the amount of phenotypic variance explained by the QTLs for OFA and defecation indicates that only a few QTLs account for the bulk of the genetic variance of these two traits (*17*).

A more conservative analysis was also used. Additive and dominance effects for each of the six markers were estimated by multiple regression analysis, and the change in R^2 (where R is the multiple correlation coefficient) due to these effects was tested for significance (ΔR^2 in Table 2). The results given in Table 2 are similar to those derived from the MAPMAKER analysis. Furthermore, multivariate analysis of variance confirmed the significance of at least three loci: chromosomes 1, 4, 12, 15, and 18 had P values less than 0.0083 (the P value is derived from 0.05 divided by six, where six is the number of putative QTLs) (*18*).

We also used MAPMAKER-QTL to calculate the LOD for simultaneously fitting the six QTLs identified for OFA. The logarithm of the likelihood that these six QTLs independently and additively contribute to the genetic variance is 38.8, accounting for 25% of the OFA variance. A similar calculation performed for the three QTLs determining defecation data gives a LOD of 12.1, accounting for 11% of the variance. Because addition of the appropriate LODs in Table 2 gives similar figures (38 and 13, respectively), it is likely that the QTLs act additively and independently.

The most parsimonious explanation of our results is that three loci (on chromosomes 1, 12, and 15) are, at least in part, the genetic basis of emotionality. Further support for this interpretation comes from consideration of the direction of effect of each QTL (Table 2). If it is true that the same QTLs determine variation in different behavioral measures, then the direction of the QTLs' effect should be as predicted from the direction of the measures' phenotypic correlation. Thus, any QTL that increases OFA should decrease defecation, increase Y maze activity, and increase the number of entries into the open arms of the EPM. Table 2 shows this to be the case for each of the QTLs that are common to the behavioral measures.

We have shown that a small number of loci with relatively large effect contribute substantially to all the genetic variance of OFA in the mouse, and that the same is probably true for defecation. We also found that activity in a Y maze is determined by three QTLs common to OFA and defecation, and that entry into the open arms of an EPM is determined by two common QTLs. Finally, the direction of each QTL's effect is as expected from the hypothesis that they are the genetic determinants of emotionality. Our data indicate that the same QTLs influence four behavioral measures and that we have localized three loci contributing to the genetic variance of emotionality.

There are cogent reasons for expecting that the genetic basis of emotionality is similar in other species and that it may underlie the psychological trait of susceptibility to anxiety in humans. The pattern of behavioral effects of anxiolytic drugs in rodents together with the results of electrophysiological and lesion experiments suggest conservation between species of a common neural substrate, probably determined by homologous genes (*7, 10*). The nature of these genes is unknown, but the discovery of QTLs determining emotionality in the mouse provides the first step toward their molecular characterization and may lead to the identification of genes responsible for human susceptibility to anxiety.

Strategies for successful positional cloning of genes responsible for complex traits will require much higher resolution mapping than achieved here (*15, 19*). In mice, the search could be narrowed by finding QTLs in homologous regions in other species and by assessment of candidate genes in the regions delimited by QTLs (none happen to lie near the QTLs reported here). Undoubtedly many genes will be involved in determining emotionality, but we have shown here that the genetic contribution to individual differences in the trait can be largely explained by variation at just three loci. Whether allelic variation occurs in the same genes in other mouse strains, or in homologous genes in other species, including humans, is unknown. Between species, evolutionary divergence of physiological and other processes may have abolished variation at one locus, or conversely introduced it at another, thus complicating the use of animal models as a basis of finding human disease susceptibility loci.

Currently there is debate about the diagnostic status of common psychiatric conditions: for instance, it is not clear to what extent anxiety disorders overlap with depression, or whether psychosis is a continuum or a set of independent entities (*20*). Genetic classification may prove a more reliable approach, with subsequent advances in both diagnosis and prognosis for many common psychiatric disorders.

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12. Open-field activity was measured under white light for 5 min in a 60-cm circular arena made of white plastic which is divided into 16 sections by photobeams that are spaced 15 cm apart. Movement is detected electronically when a photobeam is broken. Defecation was measured by the number of fecal boli produced in the open field. Entries into the open and closed arms of the elevated plus maze were scored for a 5-min period. We used the number of entries into the open arms as the measure of emotionality because, although the change in the mean percentage of open-arm entries has been used as a measure of anxiolytic and anxiogenic drug effects (*11*), the percentage itself, measured on a single occasion, does not correlate with the other behavioral measures of emotionality (correlation with OFA was 0.05 and with defecation was 0.01). However the number of open-arm entries differs significantly from the number of entries into the closed arms (*11*) and does correlate with the other measures of emotionality (0.30 with OFA and −0.15 with defecation). The Y maze consists of three arms covered by hinged lids. Photobeams divide each arm into two sections and photobeam breaks were recorded electronically for a 5-min period. Open-field defecation and entry into the open arms of the EPM were not normally distributed and were subjected to a square root transformation after the addition of 0.5 to each value (*9*).

13. The original selection experiment generated two high, two low, and two control strains (on which selection was not applied) (8). Breeding pairs for the experiment described here were derived from the first high and low strains (H_1 and L_1 respectively). DNA was extracted from spleens, diluted to a concentration of 10 ng/μl, and 50 ng used for each polymerase chain reaction (PCR). Murine microsatellites were selected that were known to distinguish C57LB/6J and BALB/cJ inbred strains (the progenitor strains for the original selection experiment) [W. F. Dietrich *et al.*, *Nat. Genet.* **7**, 220 (1994)]. Primers were obtained from Research Genetics. We tested 250 markers on the H_1 and L_1 inbred animals; 120 were found that differentiated these strains, and 84 were chosen to cover the genome with a maximum interval between markers of 30 centimorgans (cM). PCR analyses were performed in a volume of 15 μl in 96-well microtiter plates with 0.4 U of Taq polymerase, 4.0 μM deoxynucleotide triphosphate, 1× PCR buffer (Boehringer Mannheim), and 7.5 pmol of each primer. After an initial denaturation at 94°C for 4 min, reaction conditions were 30 cycles of 94°C for 45 s, 55°C for 1 min, and 72°C for 1 min. PCR products were resolved on 4% agarose gels.
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17. In addition, there is good agreement between the number of QTLs mapped for OFA and that predicted from Wright's formula for the likely number of effective factors (equivalent to the number of QTLs) [S. E. Wright, *Evolution and the Genetics of Populations: Genetic and Biometric Foundations* (Univ. of Chicago Press, Chicago, IL, 1968), pp. 373–420; W. E. Castle, *Science* **54**, 223 (1921)]. If this formula is applied to the phenotypic data from the F_2 intercross, there are 5.33 effective factors predicted for OFA. Furthermore, Wright's method also predicts a small number of QTLs for open-field defecation (2.23), in agreement with our findings.
18. When the four variables were subjected simultaneously to multivariate analysis of variance, significance levels at the marker closest to each putative QTL on the six chromosomes were as follows: chromosome 1, Wilks λ $F = 8.145$ (8 degrees of freedom), $P < 0.00001$; chromosome 4, $F = 3.601$, $P = 0.00004$; chromosome 12, $F = 4.748$, $P = 0.00001$; chromosome 15, $F = 5.531$, $P < 0.00001$; chromosome 17, $F = 2.570$, $P = 0.01$; chromosome 18, $F = 2.892$, $P = 0.004$.
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Separation of Caveolae from Associated Microdomains of GPI-Anchored Proteins

Jan E. Schnitzer,* Deirdre P. McIntosh, Ann M. Dvorak, Jun Liu, Phil Oh

In situ coating of the surface of endothelial cells in rat lung with cationic colloidal silica particles was used to separate caveolae from detergent-insoluble membranes rich in glycosyl phosphatidylinositol (GPI)–anchored proteins but devoid of caveolin. Immunogold electron microscopy showed that ganglioside G_{M1}–enriched caveolae associated with an annular plasmalemmal domain enriched in GPI-anchored proteins. The purified caveolae contained molecular components required for regulated transport, including various lipid-anchored signaling molecules. Such specialized distinct microdomains may exist separately or together in the plasma membrane to organize signaling molecules and to process surface-bound ligands differentially.

Cholesterol and glycolipids self-associate in lipid bilayers to form organized compositional microdomains (*1*). GPI-anchored and other lipid-linked proteins may preferentially partition into glycolipid microdomains that are resistant to nonionic detergent solubilization (*2–5*). GPI-anchored proteins appear to be sorted into glycolipid, detergent-resistant "rafts" in the trans-Golgi network for polarized delivery to the cell surface by caveolin-rich smooth exocytotic carrier vesicles (*3, 5–8*). On the cell surface, they are thought to reside in smooth membrane invaginations known as caveolae (*9, 10*), which are apparently also rich in glycolipids, cholesterol, and caveolin (*7, 11–14*). Antibody cross-linking of cell surface glycolipids (*1*) and GPI-linked proteins (*15*) can increase sequestration into clusters and induce cell activation (*1, 16*), apparently through lipid-anchored nonreceptor tyrosine kinases (NRTKs) (*17*). Caveolae have been implicated not only in signaling but also in transport via endocytosis, transcytosis, and potocytosis (*14, 18–21*). The physiological functions of and interrelations between caveolae, detergent-resistant microdomains, and various lipid-anchored molecules remain undefined.

Department of Pathology, Harvard Medical School, Beth Israel Hospital, 99 Brookline Avenue, Boston, MA 02215, USA.

*To whom correspondence should be addressed.

To explore the relation between GPI-anchored proteins and caveolae under conditions that avoid potential influences of antibody effectors, cell culture (*22*), and contamination from intracellular compartments, we purified the plasma membranes of rat lung endothelial cells and then subfractionated them into specific microdomains. The rat lung vasculature was perfused in situ at 10° to 13°C with a suspension of cationic colloidal silica particles, which coated the luminal endothelial cell plasma membranes normally exposed to the circulating blood. This coating created a stable pellicle that specifically marked this membrane and enhanced its density, which allowed its purification from tissue homogenates by centrifugation (*13, 23*). The silica-coated membrane pellets (P) were enriched in endothelial cell surface markers, with little contamination from other tissue components (*13, 23*).

Caveolae attached on the cytoplasmic side of the plasma membranes, opposite to the silica coating, were stripped from these membranes by shearing during homogenization at 4°C in the presence of Triton X-100. They were then isolated by sucrose density gradient centrifugation to yield a homogeneous population of biochemically and morphologically distinct caveolar vesicles (Figs. 1 and 2). As with caveolae present on the endothelial cell surface in vivo (*7, 12, 24*), these purified caveolae (V) were enriched in caveolin, plasmalemmal Ca^{2+}-dependent adenosine triphosphatase, and the inositol 1,4,5-trisphosphate receptor (*13*). In contrast, other markers present amply in P, including angiotensin-converting enzyme, band 4.1, and β-actin, were almost totally excluded from V.

The purified caveolae were not rich in GPI-anchored proteins. First, detergent extraction studies performed on P revealed differences in the ability of various detergents to solubilize caveolin and 5′-nucleotidase (5′-NT). Caveolin was partially solubilized by β-octyl glucoside, CHAPS, deoxycholate, NP-40, and SDS (but not Triton X-100), whereas 5′-NT was rendered soluble only by SDS and deoxycholate (Fig. 1A). Second, like caveolin, 5′-NT and urokinase-plasminogen activator receptor (uPAR) were enriched in P relative to the starting rat lung homogenate (H) (Fig. 1C). However, unlike caveolin, these proteins were not enriched in V; they remained almost totally associated with the resedimented silica-coated membranes stripped of the caveolae (P−V) [which contain few, if any, remaining caveolae (*13*)]. More than 95% of the signal for caveolin was detected in V, with <4% remaining in P−V. Conversely, >95% of 5′-NT and uPAR remained in P−V, with <3% present in V. Thus, these GPI-anchored proteins were neither cou-